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Preparation, Characterization and Antibacterial Activity of Metal Complexes of Mixed Ligands of Nicotinamide and Metronidazole

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Abstract: The Mn^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} complexes of mixed nicotinamide and metronidazole were prepared and characterized using solubility, melting point, conductivity measurement, CHNO (carbon, hydrogen, nitrogen, oxygen) elemental analysis, powder X-ray diffraction and infrared spectroscopy . The results of the physical and spectroscopic data confirm the formation of the complexes. For nicotinamide, coordination of the metal to the ligands occurred through the nitrogen of the pyrimidine group, while for mertonidazole, coordination took place through the nitrogen of the amine group. The antibacterial activity of the complexes was carried out against *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Klebsiella spp*, *Escherichia coli* and *Candida spp*. Only Cu(II) mixed complex had high inhibitory activity against all three bacteria species. The results of antifungal activity show that all the complexes have no inhibitory activity against the two fungi species compared with the corresponding ligands.

KEYWORDS: Nicotinamide, Metronidazole, complexes, Transition metal, Spectroscopy, Antimicrobial.

1.0 Introduction

Metal complexes have played a key role in the development of modern chemotherapy. For example, anticancer platinum drugs appear in more chemotherapy regimens than any other class of anticancer agents and have contributed substantially to the success achieved in treating cancer over the past three decades (Gielen and Tiekink, 2005). Metals can play an important role in modifying the pharmacological properties of known drugs after coordinating to such drug. The resulting pro-drugs have different physical and pharmacological properties, allowing the drug to be released in a controlled fashion or at specific location (Gonzalez *et al*., 1994). This approach may lead to the rescue of drugs that have failed because of poor pharmacology or high toxicity. For example, complexation of non-steroidal anti-inflammatory drugs to copper overcomes some of the gastric side effects of these drugs (Weder *et al*., 2002).The metal based drugs are also being used for the treatment of a variety of ailments viz. diabetes, rheumatoid arthritis, inflammatory and cardiovascular diseases as well as diagnostic agents (Underhill *et al* ., 1993; Duda *et al*., 1995).

Nicotinamide, also known as niacinamide and nicotinic amide with the IUPAC name as pyridine-3-carboxamide, is the amide of nicotinic acid (vitamin B_3, niacin). Nicotinamide is a water-soluble vitamin with molecular formula of $C_6H_6N_2O$ and is part of the vitamin B group. Nicotinic acid, also known as niacin, is converted to nicotinamide *in vivo*, and, though

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the two are identical in their vitamin functions, nicotinamide does not have the same pharmacological and toxic effects of niacin, which occur incidental to niacin's conversion. Thus nicotinamide does not reduce cholesterol or cause flushing, although nicotinamide may be toxic to the liver at doses exceeding 3 g/day for adults .In cells, niacin is incorporated into nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), coenzymes oxidation-reduction (Knip *et al*., 2000).

Some mixed ligands metal complexes have exhibited remarkable antibacterial and antifungal properties (Obaleye *et al*, 2014; Lawal *et al*., 2015). However, report on the mixed ligand complexes of Nicotinamide and Metronidazole has not been reported in literature. Hence, this paper report the preparation, characterization and biological studies of mixed metal complexes of nicotinamide and metronidazole.

2.0 Materials and Methods

2.1 Materials

2.1.1 Ligand, Drug and Microorganisms

Nicotinamide and Metronidazole were obtained from Sigma-Aldrich. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp* and *Pseudosoma aureginosa* which were obtained from Department of Microbiology, University of Ilorin. Ilorin, Nigeria.

2.2 Methods

2.2.1 Synthesis of Metal Complexes

The procedure described by Obaleye *et al*. (2014) was used to synthesize these complexes, with slight modification. One millimole of the solution of the metal salts used were dissolved in a beaker containing 10 mL of their suitable solvents and solutions of 1 millimole of the ligands were also dissolved in a beaker containing 20 mL of their suitable solvents. The solutions were mixed together and refluxed for 5 hrs but no precipitates were formed. The refluxed solution was left for 3 weeks. The precipitate obtained were washed and dried. They were kept in a sample bottle for analysis.

2.2.2 Determination of Physical Characteristics of the Complexes

The melting point test of the complexes were carried out using Gallenkamp Melting point apparatus, while the conductivity measurement were carried out in Chemistry Department, University of Ilorin with the use of HANNA instrument conductivity meter at cell constant 1.34. The infrared spectra were also carried out at the University of York, United Kingdom using FT-IR spectrophotometer.

2.2.3 Antibacterial Screening

The antibacterial activity of the ligands and the mixed complexes was carried out according to previously reported method with slight modification (Amolegbe *et al*., 2015). Seven grams of nutrient agar was weighed into a 250 mL conical flask, 250 mL distilled water (sterilized for 24 hours) was mixed with the agar and it was covered properly with cotton wool and foil paper so as to avoid contamination. The solution was then heated for 15 minutes so as to dissolve the nutrient agar and it was sterilized for 24 hours in an autoclave. The nutrient agar was then introduced into the Petri dish and was allowed to set properly to solidify. A 1 cm hole was bored at the center of the plate with the aid of a hole-borer and was allowed to remove the cracked hole so as to view the bottom of the Petri dish. The antibacterial activity of the ligands and complexes were determined at various concentrations: 200 ppm and 500 ppm. This various concentrations of complexes and ligands solutions were poured gently into the hole bored in the Petri dish and left covered. This was left in the incubator for 24 hours to allow the outgrowth of the bacteria. The zone inhibitory of the complexes and ligands were then measured and the percentage inhibition was determined.

3.0 Results and Discussion

The complexes were synthesized by the reaction of nicotinamide and metronidazole with

metals in the ratio 1:1:1. The complexes show different colours due to *d-d* electronic transition as a property of transition metals (Lawal and Obaleye., 2007). Variation in melting point may be due to the electronic configuration of each transition metal present in the complexes. The melting point of the transition metals increases as the number of valence electrons increase due to increase in the stability. Molar conductivities of the metal complexes in water, ethanol, methanol and dimethylsufoxide show non-electrolytic behaviour of the free ligands and the complexes in the solvents (Collins, 1970; Cotton *et al.*, 1999).

The result of the elemental analysis indicated that the complexes contain 1mole of Nicotinamide and Metronidazole per mole formular unit. All the complexes contain two moles of aqua ligand that are directly coordinated to the metal ion. The elemental (CHN) analyses were found to be in agreement with calculated values as shown by Table 2.

The IR spectra of the ligands and that of the complexes of their mixed ligands are shown in Table 4. In Nicotinamide, the band at 3365 cm^{-1} was assigned to **ν**(N-H) the bands at 1394 cm^{-1} was assigned to ν(C-N) both of the bands have been shifted to a different frequency in the synthesized complexes. This indicates that coordination of the Nicotinamide occurs through the nitrogen of the pyrimidine group and the Nitrogen of the amine group. Nicotinamide acts as a bidentate ligand. In the infrared spectra of Metronidazole, the band at 1125 cm^{-1} was assigned to ν (C-O). The reaction of Nicotinamide and Metronidazole with the metal salts was evidenced by the shifting of the band 3365 cm^{-1} which is assigned to ν (N-H) to a different frequency in the complexes. From the results obtained in conductivity measurements, it was observed that the complexes are non-electrolyte, which is similar to that obtained by Amolegbe *et al*. (2015)**.** The molar conductance values were measured in DMSO solution.

The occurrence of a reaction was ascertained by comparison of the XRPD patterns of the product to those of the reactants. XRPD patterns of Nicotinamide with that of Metronidazole and that of the metal complexes of their mixed ligands $[Cu(Nic)(MET)(H_2O)_2]Cl_2$ and $[Fe(Nic)(MET)(H_2O)_2]Cl_2$ as shown in Figure 1. The X-ray powder diffraction pattern of $[Cu(Nic)(MET)(H_2O)_2]Cl_2$ and $[Fe(Nic)(MET)(H_2O)_2]Cl_2$ were different from that of the ligand Nicotinamide and Metronidazole. New peaks corresponding to the complexes were observed. XRPD pattern in Figure 1 presents the X-ray powder diffraction patterns of Nicotinamide and Trimethoprim and the metal complex of their mixed ligands. The X-ray diffraction data were recorded by using Cu Kα radiation (1.5406 A^0). The relative intensity data were collected over a 2θ range of 10-40°. The Figure shows that, the XRPD of the ligands differ from that of the synthesized metal complexes of their mixed ligands, this is evidence that complexation took place. Nicotinamide shows different intensity and high peaks at 15.28^0, 18.14^0, 19.10, 23.82^0 , 24.13^0, 27.35^0 and Metronidazole at 11.78^0, 14.70^0, 19.89^0, 22.83^0, 24.30^0, 25.52^0, 33.17^0, 38.10^0, 39.40^0 and different intensity and different peaks were observed at 10.53^0, 14.82^0 , 18.42^0, 20.12^0, 23.39^0, 23.84^0, 38.18^0, 44.58^0 for $[Cu(Nic)(MET)(H_2O)_2]Cl_2$, 13.72^0, 27.10^0, 35.73^0, 44.53^0 for $[Fe(Nic)(MET)(H_2O)_2]Cl_2$. Several other new peaks were observed, this shows the formation of a new product (Lee and Wright, 1982; Tella *et al*, 2014).

In conclusion, the transition metal of Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, $Fe^{2+}$and Mn^{2+}complexes of mixed ligands have been synthesized . The complexes undergo various characterizations using solubility, conductivity, melting point, PXRD, UV/visible and infrared spectroscopy. The ligands form a chelating complex. The new alternative drugs obtained, confirmed that they are more effective than their parent ligands. From the results obtained in infrared spectra, it was revealed that the complexes are octahedral in nature. Also, the complexes were subjected into biologicall activity study against the following species: *Staphylococcus aures*, *Pseudomonas aureginosa*, *Klebsiella pneumonia* and *Esherichia coli*. The result of this shows that the complexes were active on the micro organism than the original corresponding ligands. The combined result of the physical and spectroscopic studies confirmed formation of complexes.

Table 1: Some physical properties of Nicotinamide, Metronidazole and their complexes

Ligands/ Complexes	State	Colour	Melting point (^{0}C)	Conductivity (μScm^2)
Nicotinamide	Crystalline	White	128-131	2.9
Metronidazole	Powdery	Yellow	159-161	2.6
$[Mn(NIC)(MET)(H_2O)_2].Cl_2$	Crystalline	Pink	195–197	3.2
$[Ni(NIC)(MET)(H_2O)_2].Cl_2$	Powdery	Blue	190-192	4.2
$[Cu(NIC)(MET)(H_2O)_2].Cl_2$	Crystalline	Green	310 –312	128
$[Co(NIC)(MET)(H_2O)_2].Cl_2$	Crystalline	Pink	285- 287	126
$[Fe(NIC)(MET)(H_2O)_2].Cl_2$	Powdery	Brown	340 –342	13.3
$[Zn(NIC)(MET)(H_2O)_2].Cl_2$	Powdery	White	210 – 212	6.7

Table 2: Elemental constituents of the metal complexes

Complexes	% Calculated (% Found)			
	% C	% H	% N	% O
$[Cu(NIC)(MET)(H_2O)_2].Cl_2$	31.21 (31.12)	3.71 (4.20)	15.71 (15.01)	20.79 (20.59)
$[Mn(NIC)(MET)(H_2O)_2].Cl_2$	30.72 (30.62)	3.65 (3.44)	14.93 (14.85)	23.87 (23.50)
$[Fe(NIC)(MET)(H_2O)_2].Cl_2$	30.66 (30.50)	3.65 (3.42)	14.90 (14.77)	23.83 (24.92)

Table 3: Electronic Spectra for the Free Ligands and its Complexes

Ligands/Complexes	Wavelength (nm)	Energies (cm^{-1})	Tentative Assignment
Nicotinamide	293	34130	$n \rightarrow \pi^*$
Metronidazole	332	30121	$n \rightarrow \pi^*$
$[Mn(NIC)(MET)(H_2O)_2].Cl_2$	327	30581	$n \rightarrow \pi^*$
$[Ni(NIC)(MET)(H_2O)_2].Cl_2$	394	25381	$n \rightarrow \pi^*$
$[Cu(NIC)(MET)(H_2O)_2].Cl_2$	326	30675	$n \rightarrow \pi^*$
$[Co(NIC)(MET)(H_2O)_2].Cl_2$	395	25317	$n \rightarrow \pi^*$
$[Fe(NIC)(MET)(H_2O)_2].Cl_2$	387	25840	$n \rightarrow \pi^*$
$[Zn(NIC)(MET)(H_2O)_2].Cl_2$	326	30675	$n \rightarrow \pi^*$

Table 4: **Selected infrared data (cm^{-1}) of Nicotinamide, Metronidazole (Met), and their metal complexes.**

Ligands/Complexes	*v*(N-H)	*v*(C=C)	*v*(N-O)	*v*(C-N)	*v*(M-N)	*v*(M-O)
Nicotinamide	3365	1620	-----	1394		
Metronidazole	3504	1610	1382	1226		
$[Mn(NIC)(MET)(H_2O)_2].Cl_2$	3221	1618	1369	1186	503	420
$[Ni(NIC)(MET)(H_2O)_2].Cl_2$	-------	1638	1369	1186	503	420
$[Cu(NIC)(MET)(H_2O)_2].Cl_2$	3400	1654	1331	1104	545	443
$[Co(NIC)(MET)(H_2O)_2].Cl_2$	3401	1585	1335	1095	529	420
$[Fe(NIC)(MET)(H_2O)_2].Cl_2$	3189	1591	1415	1139	520	432
$[Zn(NIC)(MET)(H_2O)_2].Cl_2$	3410	1635	1384	1142	526	427

Table 5: Antimicrobial activity of the free ligands and some of their complexes

Ligands/complexes	***E. coli***		***P.a***		***S. a***		***K.* spp.**		***Can.* spp**	
	Concentration (ppm)									
	200	500	200	500	200	500	200	500	200	500
Nicotinamide	0	0	20	0	0	30	0	0	0	0
Metronidazole	0	0	0	21	50	30	0	0	0	0
$[Mn(NIC)(MET)(H_2O)].Cl_2$	22	0	15	19	20	40	0	0	0	0
$[Cu(NIC)(MET)(H_2O)].Cl_2$	22	19	0	18	0	40	0	0	0	16
$[Co(NIC)(MET)(H_2O)].Cl_2$	16	0	0	17	0	30	0	0	0	0
$[Ni(NIC)(MET)(H_2O)].Cl_2$	0	0	0	0	0	0	0	0	0	0

E. coli = Escherichia coli; P. a = Pseudomonas aeuroginosa; S. a = Staphylococcus aureus; K. spp = Klebsiella spp; Can. spp = Candida spp

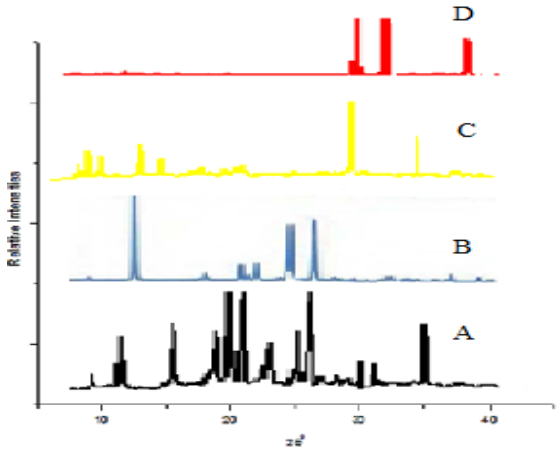

Figure 1: Powder X-ray Diffraction patterns of ligands (A) Metronidazole (B) Nicotinamide and their metal complex of mixed ligands are (C) $[Cu(Nic)(Met)(H_2O)_2]Cl_2$ (d) $[Fe(Nic)(Met)(H_2O)_2]Cl_2$

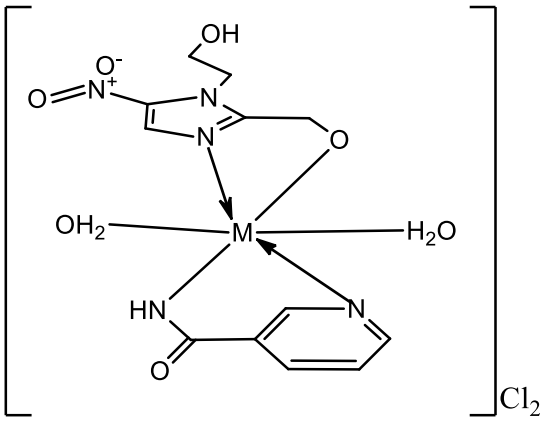

Figure 2: Proposed Structure of $[M(NIC)(MET)(H_20)_2].Cl_2$
(where M = Mn(II) , Cu(II), Fe(II), Zn (II), Co (II))

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