

# Mixed Metal Complexes of Isoniazid and Ascorbic Acid: Chelation, Characterization and Antimicrobial Activities

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## ABSTRACT

Novel mixed complexes of isoniazid and ascorbic acid have been synthesized and characterized using infrared, electronic absorption data, elemental analysis, molar conductivity, melting point, thin layer chromatography and solubility. The metal ions involved in the complex formation are  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$ . The melting points of the synthesized compounds were in the range of 170-316°C while the conductivities measured in the range of 7.67- 9.04  $\mu\text{s}$ . The infrared spectra data of these ligands and their metal complexes have revealed the bi-dentate coordination of isoniazid ligand to the metal through the oxygen of the carbonyl and nitrogen of the amine group and ascorbic acid coordinating through the carbonyl and C-2 enolic hydroxy groups. The antimicrobial studies of the synthesized compounds on micro-organisms such as: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aureginosa* and *Salmonella typhi* at a concentration of 1 mg/ml revealed that all the ligands and complexes have good antimicrobial activities. The complexes showed enhanced activities against the microorganisms. This research work is focused on the synthesis and characterization of  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  complexes of the ligand isoniazid and Ascorbic acid and their antimicrobial activities.

**Keywords:** Isoniazid, Ascorbic acid, synthesis, metal complex, antimicrobial activity.

## INTRODUCTION

Tuberculosis (TB) is a disease that produces several million deaths annually. With the appearance of multi drug resistant microbial strains of *Mycobacterium tuberculosis*, innovations in TB drug discovery and evolving strategies to bring new agents with best performance is an essential investigation <sup>1</sup>. Taking this into account, there is a pressing need to develop new and more effective antitubercular agents.

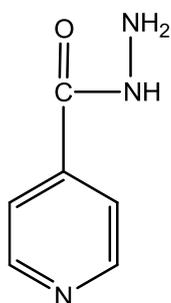
The development of more effective chemotherapeutic agent has been the main goals of coordination chemists over the years, it

has been discovered that metal complexes have enormous impact in medicine <sup>2</sup>.

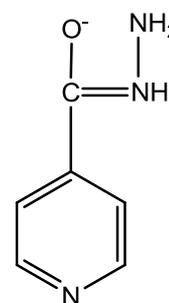
Metal ions are known to affect the action of many drugs, they play a vital role in the design of more biologically active drugs. The efficacy of the drugs on coordination with a metal is enhanced in many cases <sup>3</sup>. In several cases, the metal chelates have been found to be more antimicrobial than the chelating agents themselves <sup>4</sup>.

Isoniazid, also known as isonicotinylhydrazide (INH), is a known tuberculostatic agent used as a first-line agent

for the prevention and treatment of both latent and active tuberculosis. It forms metal chelates with many bivalent ions and these complexes have been used in the determination of the structure of isoniazid<sup>5, 6</sup>. INH is able to coordinate with metal cations through different chemical groups: heterocyclic nitrogen from the pyridine ring and/or carbonylic O and N atoms of the hydrazide group. For this versatility, it is also an interesting ligand from the chemical point of view. In order to form metal complexes, isoniazid must first form the anion (shown below) which is the Chelating species<sup>7</sup>. Vitamin C, known chemically as ascorbic acid, is an essential vitamin necessary for the treatment of scurvy<sup>8,9</sup>. Vitamin C has also been



(Isoniazid)



(Anion of isoniazid)

## MATERIALS AND METHODS

Metal salts were obtained from British House Chemical Limited, England. Ascorbic acid was obtained from Peace Pharmaceutical Company, Ilorin, Kwara State, Nigeria. Isoniazid (isonicotinylhydrazide) was obtained from Sigma Aldrich USA. Clinical isolates of the organisms were obtained from Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital (UIITH.) Ilorin, Nigeria.

### Synthesis of the Mixed Metal Complexes

The method described by<sup>12, 13</sup> were used in the synthesis of the mixed metal complexes.

cited to act as biological hydrogen carrier for redox enzyme systems in cell metabolism<sup>10</sup>. The structure of ascorbic acid has been widely studied<sup>11</sup> and was found to correspond to the enol of 3-keto-D-gulofuranolactone i.e. it has a lactone structure with an endiol group. The aim of this study is to synthesize mixed metal complexes of Isoniazid and Ascorbic acid, characterize the synthesized complexes and carry out anti-microbial analysis on the synthesized metal complexes in order to determine the complexes with higher activity against the tested organisms.

Isoniazid 0.137 g (1 mmol) was dissolved in 20 ml ethanol; 0.176 g (1 mmol) ascorbic acid was also dissolved in 20 ml distilled water. 20 ml of aqueous solution of 1 mmol of metal (M= CuSO<sub>4</sub>, CuCl<sub>2</sub>, ZnSO<sub>4</sub>, CdSO<sub>4</sub>) were added to the solution of isoniazid and Ascorbic acid in a (1:1:1) ratio. The precipitated complexes were filtered, washed, dried and kept in sample bottles for further analysis.

### Antimicrobial Activity

#### Zone of inhibition – agar well diffusion method

Some bacterial organisms (*Staphylococcus aureus*, *Pseudomonas aureginosa*,

*Klebsiella pneumonia*, and *Escherichia coli*) were collected from the Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital (UITH).

### **Preparation of Microbial Pathogens**

The overnight culture (0.2 ml) of each bacterium was dispersed into 20ml of sterile nutrient broth and incubated for about 3-5 hours to standardize the culture. A loopful of the standard cultures was used for the antimicrobial assay.

7.0 g of nutrient agar was measured into 250 ml of sterilized water and the mixture was heated for 15 minutes and placed in an autoclave to sterilize for 24 hours. The agar was poured into some sterilized petri dish and allowed to set; the bacterial species were applied on the surface of the agar using sterilized cotton swab stick. Holes were then drilled in the middle of the petri dishes by the use of sterilized cork borer with 6 mm diameter; concentration of 1mg/ml of the complexes was administered into the hole. The petri dishes were then incubated for close to 24 hours. After incubation, the effect of the complexes on various organisms was measured by calculating the zone of inhibition using a transparent ruler and the result recorded in millimeters<sup>14</sup>.

## **RESULTS AND DISCUSSION**

The analytical data obtained are shown in Table 1. Melting points were measured using open capillary tubes on an electro-thermal Gallenkamp melting point apparatus and the results showed that the

complexes exhibit high melting points, indicating a strong bonding between the ligands and metal ion. The calculated data for the elemental analysis is in good agreement with the experimentally found which confirms binding of the metal ion to ligand sites. The melting points obtained were in the range of 170- 316°C with that of the complexes higher than their parent ligands which indicate the formation of complexes<sup>15</sup>.

All the synthesized complexes were coloured and exist in powdery form indicating their polymeric nature e.g from white to green. The molar conductance values are low in the range of 7.67- 9.04  $\mu\text{s}$  suggesting their non-electrolytic nature and some degree of polarity<sup>16</sup>.

The infrared spectra of isoniazid and ascorbic acid showing the tentative assignments of the main bands of free isoniazid and ascorbic acid were compared with that of the complexes as shown in Table 2. Based on the infrared spectra data, the result of the studies supports that isoniazid and ascorbic acid act as bidentate ligands in their mode of complexation with isoniazid coordinating through the carbonyl oxygen and amine nitrogen while ascorbic acid coordination occurs through the carbonyl and C-2 enolic hydroxy groups. Complexation through the C2 enolic hydroxy group is possible because of its acidic nature as suggested by previous investigators<sup>17</sup>.

**TABLE I: Analytical data of some mixed isoniazid– ascorbic acid drug metal complexes:**

Ligand/ Complexes	Elemental Analysis C H N (calc)%	Color	% yield	Melting point (°C)	Conductivity $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$	TLC ( $R_f$ )
Isoniazid	-	White	-	170-172	-	0.75
Ascorbic acid	-	White	-	190-192	-	0.47
Zn(IND)(ASC)SO <sub>4</sub>	48.32(47.03) 5.41(5.49) 14.09(13.99)	Cream	65	280-282	8.56	0.59
Cu(IND)(ASC)Cl <sub>2</sub>	47.88(47.60) 5.72(5.91) 14.78(15.10)	Gold	74	220-222	9.04	0.60
Cu(IND)(ASC)SO <sub>4</sub>	48.90(48.63) 5.60(5.88) 14.86(14.50)	Dark Green	69	250-252	8.85	0.40
Cd(IND)(ASC)SO <sub>4</sub>	49.00(48.88) 5.33(5.01) 13.87(14.05)	Cream	70	314-316	8.20	0.64

TLC: Thin layer chromatography

**TABLE II: Selected infrared spectra for some isoniazid- ascorbic acid and its metal complexes.**

Complexes/Ligands	$\nu\text{N-H}$	$\nu\text{O-H}$	$\nu\text{C=N}$	$\nu\text{C-H}$	$\nu\text{C=O}$	$\nu\text{C-O}$	$\nu\text{M-L}$
Isoniazid	3294	-	1554	3024	1653	-	-
Ascorbic acid	-	3559	-	-	1743	1002	-
Zn(IND)(ASC)SO <sub>4</sub>	3110	3120	1490	3155	1680	1121	-
Cu (IND)(ASC)Cl <sub>2</sub>	3491	3520	1550	3051	1599	1034	-
Cu(IND)(ASC)SO <sub>4</sub>	3251	3387	1580	3057.	1650	1049	596
Cd(IND)(ASC)SO <sub>4</sub>	3302	3465	1557	3123	1654	1064	611

**TABLE III: Selected uv-visible spectra for some isoniazid- ascorbic acid drug metal complexes.**

Ligands/ complexes	Wavelength(nm)	Wavenumber (cm <sup>-1</sup> )	Assignment
Isoniazid	211	47393	$\pi - \pi^*$
	295	33878	n- $\pi^*$
Ascorbic acid	226	44247	$\pi - \pi^*$
	274	36496	n- $\pi^*$
	430	23255	n- $\pi^*$
Zn(IND)(ASC)SO <sub>4</sub>	265	37735	CT
Cu (IND)(ASC)Cl <sub>2</sub>	430	23255	MLCT
	676	14792	2Eg→2T <sub>2g</sub>
Cu(IND)(ASC)SO <sub>4</sub>	622	16077	MLCT
	661	15128	2Eg→2T <sub>2g</sub>
Cd (IND)(ASC)SO <sub>4</sub>	208	48076	CT

IND=Isoniazid    ASC=Ascorbic acid

**TABLE IV: Antimicrobial activities of some mixed isoniazid - ascorbic acid metal drug complexes**

**ZONE OF INHIBITION (mm) 1 mg/ 1 ml**

Complexes/Ligands	<i>E.Coli</i>	<i>Staph. Aureus</i>	<i>Kleb. Pneumonia</i>	<i>Pseudo. Aureginosa</i>
<b>Isoniazid</b>	1.0	0	0	1.0
<b>Ascorbic acid</b>	NA	2.0	NA	NA
<b>Zn(IND)(ASC)SO<sub>4</sub></b>	0.9	0	5.0	0
<b>Cu (IND)(ASC)Cl<sub>2</sub></b>	2.0	8.2	16.0	0.5
<b>Cu(IND)(ASC)SO<sub>4</sub></b>	4.2	8.0	0.7	5.8
<b>Cd (IND)(ASC)SO<sub>4</sub></b>	6.0	3.0	14.5	2.1

NA=No activity

The band at 1653 cm<sup>-1</sup> in the spectrum of the ligand (isoniazid) is a characteristic of the C=O. This band underwent a shift to 1721 cm<sup>-1</sup>, 1680 cm<sup>-1</sup>, 1599 cm<sup>-1</sup>, 1650 cm<sup>-1</sup>

and 1654 cm<sup>-1</sup> in the spectra of Cu(II), Zn(II),Cu(II) and Cd(II) complexes, showing the participation of the carbonylic oxygen ion in coordination. The movement of the band 3294 cm<sup>-1</sup> to lower wave

numbers suggests the involvement of the amino nitrogen in the coordination to the metal ions.

The C=O on the ascorbic acid moiety also observed at  $1743\text{ cm}^{-1}$  underwent a shift to a wavelength of  $1721\text{ cm}^{-1}$ ,  $1680\text{ cm}^{-1}$ ,  $1599\text{ cm}^{-1}$ ,  $1650\text{ cm}^{-1}$  for Cu(II), Zn(II), Cu(II) and Cd(II) in the complex also showing a potential binding site from the ascorbic acid ligand. The (O-H) from the ascorbic acid ligand observed at  $3559\text{ cm}^{-1}$  underwent a shift in the metal complex to a frequency of  $3331\text{ cm}^{-1}$ ,  $3120\text{ cm}^{-1}$ ,  $3520\text{ cm}^{-1}$ ,  $3387\text{ cm}^{-1}$  and  $3465\text{ cm}^{-1}$  for Cu(II), Zn(II), Cu(II) and Cd(II) complexes indicating the involvement of (O-H) hydroxyl oxygen atom in coordination to the central metal atoms.

Based on the infrared spectra data, the result of the studies supports that isoniazid and ascorbic acid act as bidentate ligands with isoniazid coordinating through the carbonyl oxygen and amine nitrogen while ascorbic acid coordination occurs through the carbonyl and C-2 enolic hydroxy groups.

The UV-Vis spectrum of the ligand (isoniazid) shows peaks at 211 nm ( $47393\text{ cm}^{-1}$ ), 295 nm ( $33878\text{ cm}^{-1}$ ) assigned to ( $\pi\rightarrow\pi^*$ ), and ( $n\rightarrow\pi^*$ ) electronic transitions. The spectrum of the free ligand ascorbic acid, exhibits absorption peak at 226 nm ( $44247\text{ cm}^{-1}$ ) and an intense peak at 274 nm ( $36496\text{ cm}^{-1}$ ), which is assigned to ( $\pi\rightarrow\pi^*$ ), and ( $n\rightarrow\pi^*$ ) transition respectively. These transitions have been attributed to intra ligand transfer. The (U.V- Vis) spectrum of [Cu(IND)(ASC)]Cl<sub>2</sub> and Cu(IND)(ASC)SO<sub>4</sub> exhibited two peaks, the first peak at (430 nm)( $23255\text{ cm}^{-1}$ ) and (622nm)( $16077\text{ cm}^{-1}$ ) is due to the (LMCT), while the second weak peaks at (676nm)( $14792\text{ cm}^{-1}$ ), (661nm)( $15128\text{ cm}^{-1}$ ) which is assigned to ( $2E_g\rightarrow 2T_{2g}$ ) transition in an octahedral geometry<sup>18,19,20</sup>.

The Zn(II), and Cd(II) complexes did not display any peak in the visible region, no

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ligand field absorptions band was observed, therefore the bands appeared in the spectra of two complex at 265nm( $37735\text{ cm}^{-1}$ ) and 208nm( $48076\text{ cm}^{-1}$ ) could be attributed to the Ligand to Metal (L→M) charge transfer transition which is compactable with tetrahedral structure for Zn(II) complex<sup>21</sup>.

From the UV visible data available, tetrahedral geometry have been assigned to some of the prepared complexes. An interesting discovery is that the tuberculostic activity of isoniazid is increased tenfold in the presence of copper ions<sup>22</sup>.

## CONCLUSION

The novel mixed isoniazid –Ascorbic acid complexes were found to be very stable. The infrared spectra result revealed that isoniazid and ascorbic acid act as bidentate ligands in their mode of complexation with isoniazid coordinating through the carbonyl oxygen and amine nitrogen while ascorbic acid coordination occurs through the carbonyl and C-2 enolic hydroxy groups. The result of the antimicrobial studies showed that all the mixed complexes showed higher activity compared to the parent ligand isoniazid and ascorbic acid. The metal complexes showed enhanced activities compared to the free ligands, the copper complexes in all the cases studied proved to be a better antimicrobial agent compared to the ligands and other metal complexes.. The presence of several potential donor sites, e.g. heterocyclic nitrogen from the pyridine ring, carbonylic Oxygen and N atoms of the hydrazide group, and amide nitrogen atoms make them versatile complexing agent with metal ions.

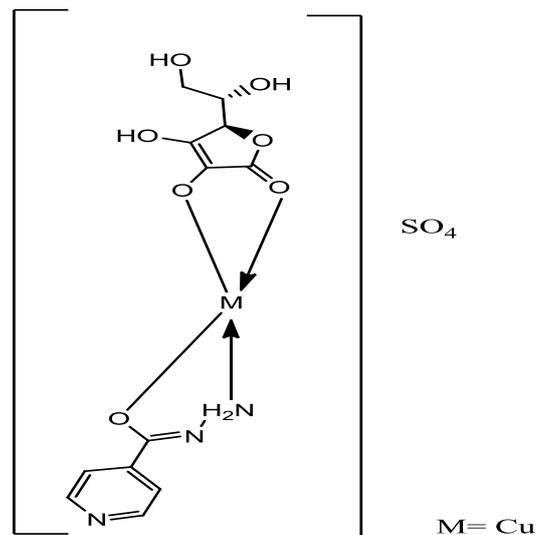
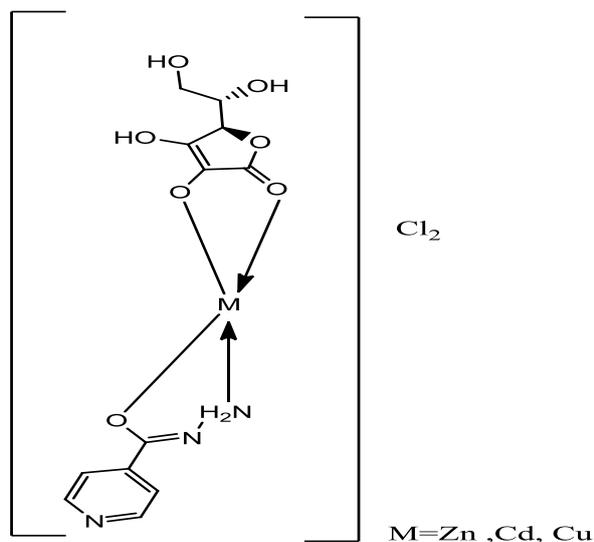
## Proposed Structure for the Metal Complex

Based on these data of structural investigation on the complexes prepared,

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the following structure is suggested

(tetrahedral geometry).



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