Chelation, Characterization and Antimicrobial Screening of Some Mixed Antituberculosis–Vitamin Metal Drug Complexes

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Abstract

In this study, mixed Zn(II), Cu(II), Co(II) complexes of Isoniazid and vitamin C were synthesized and characterized by melting point, conductivity measurement, Fourier Transform Infra-red (FT-IR), Atomic Absorption Spectroscopy and elemental analyses. The bonding nature of the mixed parent ligands and the structure of the complexes were based on analytical and spectroscopic techniques. The complexes were proposed to have the formula $[M_1L_1L_2(Cl_2)]$, $[M_2L_1L_2(SO_4)]$, $[M_3L_1L_2(Cl_2)]$ where M_1 = Zn(II), M_2 = Cu(II), M_3= Co (II) L_1= Isoniazid and L_2 = Vitamin C. The infra-red data relate to the results of the most informative and indicative region. The result of spectra data confirmed that the two ligands were bidentate in their mode of coordination with the metal ions. The complexes are in octahedral geometry and the molar conductance was found to be non-electrolytic in nature. Antimicrobial test was carried out against four bacterial species namely *Bacillus subtilus*, *Staphylococuss aureus*, *Escherichia coli* and *Pseudomonas aeroginosa*. All the complexes showed higher antibacterial activity at the same concentration against the microorganisms used compared to the ligands.

Keywords: Coordination, Spectra, Evaluation, Bidentate.

1.0 Introduction

Tuberculosis is a worldwide disease that causes millions of death annually [1]. Due to this critical situation, discovery of tuberculosis drug is a research priority aim at producing new agents with best performance [2].The development of sensitive chemo-sensors is an active field of research in recent years because of their potential application in clinical biochemistry as well as analytical chemistry and environmental science [3-6]. Synthetized metal complexes with Ligand of great biological importance have immense interest in bio-inorganic chemistry [7, 8].Silver complexes with α-hydrocarboxylic acids have been reported to be effective antimycobaterial compounds, and also a potential candidate for antiseptic or disinfectant products [9]. Transition metals play important roles in human. They are used by the living cells as cation and are strictly regulated because some are harmful when used in excess [10, 11]

Isoniazid is a first line anti-tuberculosis drug for the treatment of tuberculosis [12]. In the discovery of new alternative drug metal complexes, the modification of existing drug by coordination to a metal ion has attracted considerable attention in recent years. From previous research, it has been reported that the development of active metal drugs are under investigation since years back. Inorganic compounds have a great responsibility in medicine [13, 14]. It has been reported that antituberculosis drugs are used together with vitamin B in order to combat the toxic side effect of the drug. Hence, in order to reduce the side effect of isoniazid, it would be desirable to synthesize some novel mixed complexes of isoniazid with vitamin C which will be more effective than their parent drugs. Thus, this article reports the chelation, characterization and antimicrobial screening of mixed metal complexes of isoniazid–vitamin C.

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2.0 Materials and Methods

2.1 Chemicals and Reagents

All chemicals and solvents used were of analytical grade and obtained from Sigma-Aldrich without any further purification. Isoniazid was obtained from Sigma-Aldrich Company, USA while Vitamin C was obtained from Rajrab Pharmaceutical Company, Ilorin, Nigeria. The metal salts used were obtained from Department of Chemistry, University of Ilorin, Ilorin, Nigeria.

2.2 Synthesis of Complexes

The procedure developed by Tella [14] was adopted in the synthesis of complexes. Isoniazid (1 mmol) was dissolved in 20 ml of methanol and 1 mmol of Vitamin C was also dissolved in 20 ml of ethanol. The ligands solutions were added together and mixed with 1 mmol of metal salts solution (M = $ZnSO_4.7H_2O$, $CuCl_2.2H_2O$, $CoCl_2.6H_2O$) in 20 ml each of distilled water. Precipitation occurred immediately and the complexes thus separated were filtered and washed with mixed methanol and ethanol and later dried in a desiccator for 7 days.

2.3 Characterization of Complexes

The melting point of all the complexes was determined using Gallen Kamp melting point apparatus. The conductivity test was carried out and recorded using Hanna instruments and EC 214 Conductometer Bridge. Infra-red spectra of the ligands and complexes were recorded using FT-IR spectrophotometer in the range of 4000 – 400 cm^{-1}. Elemental analysis of both the ligands and the complexes was carried out using Perkin Elmer 204C micro- analyser.
The antibacterial studies of the synthesized compounds were carried out against their parent drugs. Molecular weights of the complexes were determined using Rast's camphor method. Analysis of the metal content was determined using Atomic Absorption Spectrometer (Thermo S-series) and reported at Obafemi Awolowo University, Ile Ife, Nigeria. The molecular weights of the metal drug complexes were determined using the formula:

$$M = \frac{K \times w \times 100}{\Delta T \times W}$$

Where K = Molecular depression content of camphor, w = Weight of the complexes taken, W = weight of camphor (39.7) ΔT = Depression of the melting point.

2.4 Antibacterial Activity

Screening of antibacterial activity of the ligands and their metal complexes was done following the procedure adopted by Obaleye [15]. The media used for the antibacterial study was nutrient agar. The culture media was prepared by dissolving 28 g of the nutrient agar in 1 litre of distilled water. The solution was shaken to allow proper mixing and heated to dissolve the agar completely. After heating, the conical flask was covered with cotton wool and aluminium foil. It was autoclaved at a temperature of 121^oC for 15 minutes. The molten agar was then poured into petri dishes and allowed to solidify. The plates were inoculated with 24 hours old culture of the organisms namely *Baccilus subtilus*, *Staphylococuss aureus*, *Escherichia coli* and *Pseudomonas aeroginosa*. Sterilized cork hole borer was used to make hole in the inoculated agar plates. Concentrations of 20 ppm and 40 ppm of both the ligands and metal salts solutions were introduced into the agar in the dishes. The petri dishes were covered and incubated at 37^oC for 24 hours after which the presence of zone of inhibition was observed around the hole of the agar plates.

3.0 Results and Discussion

The analytical properties of the complexes are presented in Table 1. All the complexes exhibited the colour of their metal salts, which indicate partial evidence of complexation. $[Cu(ISO)(Vit.C)Cl_2]$ melts within the range of 243 – 245^oC while $[Zn(ISO)(Vit.C)SO_4]$ and $[Co(ISO)(Vit.C)Cl_2]$ melt above 360^oC. The molar conductance values for the complexes are in the range of 1.90 – 2.00 $\Omega^{-1}cm^2mol^{-1}$. From the results, it was observed that the complexes are non-electrolyte. The theoretical metal content (% yield) and molecular weight (g) of the complexes (Table 2) were found to compete favourably to the experimental values. The elemental analyses of the complexes presented in Table 3 are in agreement with 1:1 metal to ligand stoichiometry for the complexes. They are consistent with the theoretical results from the empirical formula of each compound.

Table 1: Analytical Properties of the Complexes

Ligands/Complexes	Colour	Melting Point (^oC)	Conductivity $\Omega^{-1}cm^{-2}mol^{-1}$
Isoniazid (ISO)	White	176-178	-
Vitamin C (Vit. C)	White	198-200	-
[Zn(ISO)(Vit. C)SO_4]	White	>360	1.90
[Cu(ISO)(Vit.C)Cl_2]	Deep Green	243-245	2.46
[Co(ISO)(Vit. C)Cl_2]	Pink	>360	2.00

Table 2: Molecular weight and Atomic Absorption Spectroscopy of the Complexes

Ligands/Complexes	Percentage yield (%)	Experimental Molecular Weight	Theoretical Molecular Weight	% Metal Expected	% Metal Present
Isoniazid	-	-	-	-	-
Vitamin C	-	-	-	-	-
[Zn(ISO)(Vit. C)SO_4]	47.50	474	475	13.71	13.39
[Cu(ISO)(Vit.C)Cl_2]	38.11	447	476	14.32	14.98
[Co(ISO)(Vit.C)Cl_2]	71.00	442	441	13.35	13.34

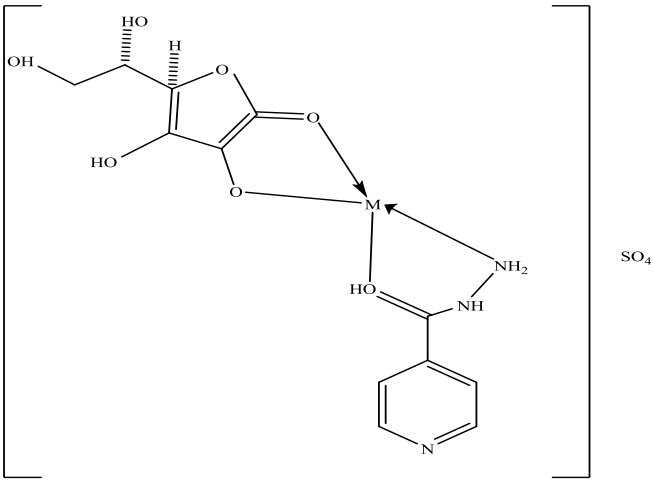
Table 3: Elemental Analysis of the Complexes

Ligands/Complexes	Experimental (Calculated)			
	% C	% H	% N	% M
Isoniazid	52.55 (52.16)	5.11 (5.32)	30.66 (30.00)	-
Vitamin C	39.97 (40.91)	4.00 (4.55)	-	-
[Zn(ISO)(Vit. C)SO_4]	30.00 (30.38)	3.30 (3.16)	8.85 (8.86)	13.39 (13.71)
[Cu(ISO)(Vit.C)Cl_2]	32.34 (32.21)	3.45 (3.36)	9.09 (9.40)	14.98 (14.32)
[Co(ISO)(Vit. C)Cl_2]	32.67 (32.58)	3.76 (3.39)	9.35 (9.50)	13.34 (13.35)

The infra-red spectra of the complexes were compared with those of the ligands within 4000 band– 400 cm^{-1} (Table 4). Based on this studies, tetrahedral geometry has been proposed for [Zn(ISO)(Vit.C)SO_4] complexes (Figure 1) while [Cu(ISO)(Vit.C)Cl_2] and [Co(ISO)(Vit.C)Cl_2] showed octahedral geometry (Figure 2). The band at 1666 cm^{-1} substituted to ν (C=O) of isoniazid was shifted to 1741 cm^{-1} in the Co(II) and Cu(II) complexes due to coordination through this point. This is in agreement with the interaction through the oxygen of the carbonyl. In all the complexes, Isoniazid coordinates through the oxygen of the carbonyl group and nitrogen of the amine group. However in Vitamin C, coordination occurs through the oxygen of carbonyl group and hydroxyl group. The band set around 3300 cm^{-1} due to V(N-H_2^+) which is observed in the ligand is also present in all the complexes with high shift due to hydrogen bonding indicating that the protonated state of the Ligand remain upon complexation [14].

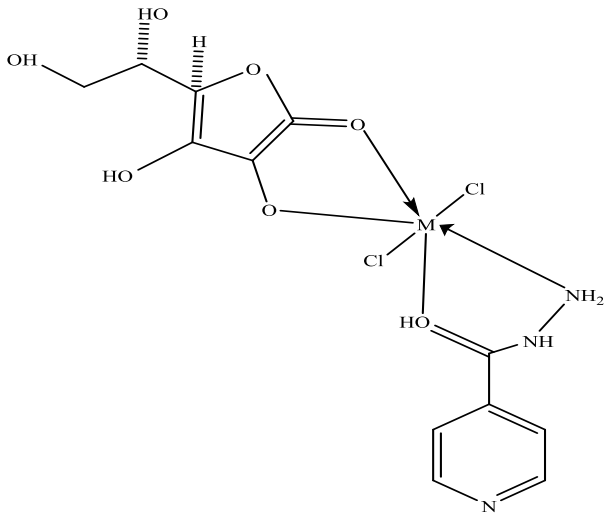
Table 4: Infra-red Spectra of the Complexes

Ligands/Complexes	ν (C=O)	ν (N-H_2^+)	ν (C-O)	ν (O-H)
Isoniazid	1666	3300	-	-
Vitamin C	1658	-	1116	3550
[Zn(ISO)(Vit. C)SO_4]	1653	3462	1103	3263
[Cu(ISO)(Vit.C)Cl_2]	1741	3400	1039	3250
[Co(ISO)(Vit. C)Cl_2]	1741	3450	1053	3200



M = Zn

Fig. 1: Proposed structure of the mixed complexes of Zn(II)



M = Cu, Co

Fig 2: Proposed Structure of the mixed complexes of Cu(II) and Co(II)

The analysis of FT-IR spectra of the Ligands and the complexes give details on the mode of coordination between the Ligands and the metal IR spectra [3]. From the spectra of the Copper- Sulphate complexes, it reveals that a broad band in the region 3250 cm^{-1} due to stretching vibration of O-H group [15]. The chloride ion present in [Cu(ISO)(Vit. C)Cl_2] and [Co(ISO)(Vit.C)Cl_2] were tested using $AgNO_{3(aq)}$. It was observed that no precipitation of silver halide occurred. This shows that there is presence of chloride ion inside the coordination sphere of the synthesized complexes [14], and the test for sulphate ion carried out on [Zn(ISO)(Vit.C)SO_4] was positive due to coordination of the ion to the metal [14]. Based on the molecular weight data, it was revealed that the complexes are in good condition since the experimental values are compared with the theoretical values.

The antibacterial activities of the ligands and their complexes against four bacteria species namely *Baccilus subtilus*, *Staphylococuss aureus*, *Escherichia coli* and *Pseudomonas aeroginosa* were presented in Table 5. From the result, it was observed that all the complexes showed high inhibition against the organisms. This indicates that the coordination could have the ability of the complexes to cross a cell membrane [16-18]. The ligands had no effect on the organism especially Vitamin C.

Table 5: Antimicrobial Activity of the Complexes

Ligands/Complexes	***Baccillus subtilis***		***Staphylococcus Aureus***		***Escherichia coli***		***Pseudomonas aeruginosa***	
Concentration	20 ppm	40 ppm	20 ppm	40 ppm	20 ppm	40 ppm	20 ppm	40 ppm
Isoniazid	0	0	23	24	0	0	0	0
Vit.C	0	0	0	0	0	0	0	0
[Zn(ISO)(Vit. C)SO_4]	30	14	20	11	8	5	15	7
[Cu(ISO)(Vit.C)Cl_2]	10	4	6	4	12	5	31	15
[Co(ISO)(Vit. C)Cl_2]	30	14	20	11	8	5	15	7

4.0 Conclusion

Based on the spectral data obtained in this study, octahedral geometry has been proposed for all the complexes. The results of both the physical and spectroscopic data confirm that the two ligands are chelating agents. All the complexes also showed higher antibacterial activity compared to those of the ligands.

5.0 References

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