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SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF Ni (II), COMPLEX DERIVED FROM CLOXACILLIN AND CEPHALEXIN

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Abstract

A new metal complex of Ni (II) with cloxacillin and cephalexin (mixed ligands), [Ni (Clox) (Cep)Cl_2] was synthesized in 1:1:1 ratio. The complex was characterized for its physicochemical properties and spectral studies. From the IR and electronic spectral studies, the spectra of the complex were found to be different from that of the ligands suggesting the formation of coordination compound. The ligands coordinated with the Ni (II) ion through the carbonyl group (C=O) of the carboxylate and the carboxyl groups of the amide in both the cloxacillin and cephalexin antibiotics. This has made the complex to assume octahedral geometry. The complex has shown good antibacterial activity. It is therefore recommended among others that; pharmaceutical companies and other researchers should exploit these findings to process the complex further to produce potent antimicrobial drugs.

Keywords: *Ligands, Complex, Synthesis, Antibacterial activity, cloxacillin, cephalexin*

Introduction

The ever-increasing spread of bacterial infection with antibacterial drug resistance has become a matter of global concern in the modern times. Therefore, such serious infections leading to high mortality rate has necessitated search for classes of effective drugs for curing bacteria with minimum toxicity [9].

It was reported that among the synthesized Co (II) and Ni (II) coordination compounds of thiosemicarbazone has shown relatively promising results against mycobacterium tuberculosis. Also, antimicrobial studies of the ligands and complexes indicate that the activity increases on chelation.[12]

Synthesis and characterization of mixture of cephalexin (cep) and amoxicillin (amx) in 1:1 mole ratio interacted with transition metal ions to give [M(cep) (amx)]. $3H_2O$ complexes in 1:1:1 mole ratio, where M = Co (II), Ni (II), Zn (II), and Fe (III). The complexes were characterized by physicochemical and spectroscopic analysis. The UV/Vis spectral data suggested an octahedral[13] geometry for Co (II), Ni (II), and Fe (III) structure, while Zn (II) complex adopted tetrahedral geometry. The IR spectral data shows that the ligands coordinated by metal ions through their υ (NH.), υ(COO), and υ (C = O) functional group respectively due to the similarity in the structures.

Many approaches (like solution-based and solid-state) were published for the synthesis of ligand-metal complexes and the various spectroscopic analyses such as IR spectroscopy, UV-visible spectroscopy, NMR spectroscopy have been used to elucidate the properties of complexes [6]. Various transition metals with drugs molecules complexes show wide range of pharmaceutical activities, that place them in several biochemical processes and antimicrobial agents.

In this article, the solution-based synthesis of a new transition metal complex of mixed antibiotics (Cloxacillin and Cephalexin), i.e. [Ni (Clox)(Cep)Cl_2] was obtained by refluxing the antibiotics and the inorganic salt of $NiCl_2$ and also to determine the antimicrobial activity of the complex.

MATERIALS AND METHODS

All the reagents were of analytical grade and used without any further purification. The active pharmaceutical ingredients (API), cephalexin, and cloxacillin as well as Nickel (II) chloride were obtained from Sigma Aldrich Nigeria. All the glassware and other apparatus used for the synthesis of the complex were obtained from Department of Chemistry Laboratory, Federal University Dutse.

SYNTHESIS OF METAL (II) COMPLEXES

An aqueous (20 ml) solution of the antibiotics (10 mmol of cloxacillin trihydrate (Clox) and 10 mmol of cephalexin monohydrate (Ceph) were mixed in 1:1 mole ratio. The

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solution of the mixed antibiotics was further mixed with 10 ml aqueous solution of $NiCl_2.6H_2O$, in 1:1:1 mole ratio. The reaction mixture was refluxed for 5 hours on a hot plate magnetic stirrer at 50°C. The volume of each solution was concentrated to half of the initial volume. The product obtained was allowed to cool, washed with water, diethyl ether, and then dried in a vacuum over $CaCl_2$ [13].

Physical properties of the synthesized complex

Based on the method by [13], the melting point and temperature decomposition were carried out using Stuart Melting Point apparatus (SMP 10), solubility of the ligands and complex were determined in different solvents ranging from polar to non-polar such as distilled water, methanol, ethanol, benzene, CCl_4, diethyl ether, acetone, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) and the molar conductivity was carried out using conductivity meter of model DDS-307 in $1x10^{-3}$ at Central Laboratory Bayero University Kano.

Magnetic Susceptibility Measurement

$$n = \frac{X_i}{1-X_i}$$

Where n = number of coordinated ligands

X_i = mole fraction of maximum absorbance

Magnetic susceptibility measurement of the complexes was recorded using Magnetic Susceptibility balance of Sherwood Scientific Cambridge UK. The prepared metal complex was introduced into the balance's capillary tube up to a given mark and the reading recorded using the magnetic susceptibility balance. The formula below was used to calculate the magnetic susceptibility (Xg) [2].

$$Xg = \frac{C \times L(R-Ro)}{10^9 M}$$

Spectral studies

FTIR spectra were recorded using Fourier Transform Infrared Spectrophotometer of Shimadzu of 4000-400 nm wavelength at the National Research Institute for Chemical Technology Zaria. The IR spectra were recorded on the IR spectrophotometer in solid state [5].

UV-Vis Spectral studies

Perkin Elmer Lambda 35 UV-Visible Spectrophotometer of 200-700nm wavelength was used for UV-absorbance measurement and the results were recorded at the Central Laboratory Bayero University Kano. The UV -Vis spectra were determined by UV- visible spectrometer using DMF as solvent [2].

Elemental Analysis (C.H.N.S)

Elemental analysis technique was recorded on Euro EA Elemental Analyser (2000°C) at Central Laboratory Umaru Musa Yar'Adua University Katsina. Also, the metals contents of the complexes were measured using flame atomic absorption spectroscopy at the same laboratory.

Antimicrobial Activity Method

Sensitivity discs were punched from Whatman no. 1 filter paper, sterilized in bijou bottles by autoclaving at 121°C for 15 minutes. Sensitivity discs were prepared by weighing 0.008 mg of the extract or fraction and serial doubling dilution in DMF followed by placing the improvised paper discs in the solution such that each disc took up 0.01mL to make the disc potency of 500μg, 1000μg, 2000μg, and 4000μg. Standardized inoculate of each isolate were swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes and discs of the extracts and standard antibiotic (Ciprofloxacin 500μg). The plates were inverted and allowed to stand for 30 minutes for the extract to diffuse into the agar after which the plates were incubated aerobically at 35°C for 18 hours. This was followed by measurement of zone inhibition formed by the test organisms around each of the extract and standard antibiotic discs [15].

RESULTS AND DISCUSSION

Table 1: Physical Properties of the Antibiotics and their Metal (II) Complex

Compound	Molecular Formula	Molar Mass (g/mol)	Colour	Melting Point (°C)	Decomposition Temperature (°C)
Cloxacin	$C_{19}H_{18}ClN_3O_5S$	435.9	White	170	___
Cephalexin	$C_{16}H_{17}N_3O_4S$	347.4	White	197	___
[Ni(Cep)(Clox)Cl_2]	$C_{35}H_{35}Cl_3N_6O_9S_2Ni$	912.19	Pale green	___	262

The synthesis of Ni (II) inorganic complex was completed within 1 to 2 hours. The mixed antibiotic ligands (cloxacillin and cephalexin) produced pale green [Ni (Cep) (Clox) Cl_2] complex. The colour is due to d-d transition and reactivity between the ligands and the metal ion which is similar with results reported by [4].

The complex exhibit higher melting decomposition temperature than the individual free ligands as presented in Table 1. Cloxacillin melted at 170°C, Cephalexin melted at 197°C while their complex decomposed at 262°C. The higher value of the mixed complex

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provides evidence of complexation of the ligands to the metal ion. It also revealed the more stable nature of the complex. These values are in agreement with similar metal (II) complexes reported by [13] and [3].

Table 2: Solubility of the Antibiotics and their Metal (II) Complex

Compound	Distilled Water	CCl_4	Benzene	Diethyl ether	Acetone	Ethanol	Methanol	DMF	DMSO
Cloxacillin	S	IN	IN	SS	S	IN	IN	S	S
Cephalexin	S	IN	IN	SS	S	IN	IN	S	S
$[Ni(Cep)(Clox)Cl_2]$	SS	IN	SS	SS	SS	IN	IN	S	S

As presented in Table 2, cloxacillin and cephalexin are both soluble in distilled water, acetone, DMF, and DMSO, slightly soluble in diethyl ether while insoluble in CCl_4, benzene, ethanol, and methanol respectively. Ni (II) complex prepared is insoluble in CCl_4, ethanol, and methanol, slightly soluble in distilled water, benzene diethyl ether, and acetone, and soluble in DMF and DMSO. It can be realized that the metal (II) complex is either insoluble or slightly soluble in some organic solvents such as ethanol, methanol, diethyl ether, and acetone but soluble in DMF and DMSO. The values would suitably be used to conduct conductivity test. This is because polar compounds are expected to be soluble in polar solvents and vice-versa (like dissolves like) which is in agreement with the results reported by [15] and [8].

Table 3: The IR Spectra (4000-400 cm^{-1}) of Antibiotics and their Metal (II) Complex

Compound	υ(O-H)	υ (C = O) of CO_2	υ (C = O) of amide	M – O	M – Cl
Cloxacillin	3677.85	1689.66	1771.53	--------	------
Cephalexin	3392.90	1687.77	1757.21	----------	-------
$[Ni(Cep)(Clox)Cl_2]$	3398.69	1639.55	1755.28	466.79	854.49

The IR spectra data of the free ligands and their complex as presented in Table 3 were discussed and compared with each other to predict the coordination mode and the expected final shape depending on ligand behaviour and other coordination molecules such as chloride ions. Based on the spectra, the absorption bands at 3677.85 cm^{-1} in cloxacillin and 3392.90 cm^{-1} in cephalexin ligands is attributed to O–H stretching which is shifted averagely to low frequency of 3398.69 cm^{-1} in the Ni (II) complex. This is in agreement with coordination through the oxygen in the OH of the carboxylic group in both the two ligands. The bands at the of 1689.66 cm^{-1} and 1687.77cm^{-1} are assigned to the carbonyl group (C=O) of carboxylate groups in cloxacillin and cephalexin ligands respectively which were shifted to different frequencies of 1639.55cm^{-1} in $[Ni (Cep)(Clox)Cl_2]$, indicating occurrence of complexation through carboxyl of the carboxylate group with the metal ion. The bands at 1771.53cm^{-1} and 1757.21cm^{-1} are assigned to the carboxyl group of amide in the ligands respectively which clearly differs from 1757.21cm^{-1} in the complex. The shifting of values in the complex shows the complexation through the carboxyl of amide with metal ion under investigation as reported by Waziri *et al.*, [12]. The complex $[Ni (Cep)(Clox)Cl_2]$ indicated new bands at 466.79 and 854.49 cm^{-1} which are assigned to υ (M – O) and υ (M – Cl), which could not be traced in the free ligands proves the formation of the new compound as reported by [6].

Table 4: Electronic Spectra, Conductance in DMF Solvent and Magnetic Moment (B.M) for Synthesized Metal Complex with its suggested geometry

Ligands/Complex	Electronic spectra			Conductance (Ω^{-1} cm^2mol^{-1})	Magnetic moment (BM)	Suggested geometry
	Wavelength (nm)	Energy (cm^{-1})	Transition			
Cloxacillin	202	48309	$n - \pi^*$			
	207	49505	$\pi - \pi^*$			
	239	41841	CT band			
Cephalexin	220	45455	$n - \pi^*$			
	233	42918	$\pi - \pi^*$			
$[Ni (Cep)(Clox)Cl_2]$	221	45249	MLCT	20.00	2.98	Octahedral
	230	43478	$^3A_2g \rightarrow {}^3T_2g$			
	237	42194	$^3A_2g \rightarrow {}^3T1_g$			

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The molar conductance in $\mathbf{1 \times 10^{-3}}$ DMF is presented in Table 4. Conductivity measurements have been used in elucidating the structure of complexes within the limits of their solubilities in a given solvent as reported [1] which suggested that the values of less than < 60 in DMF are considered to be non-electrolytes. The Ni (II) complex has molar conductance of 20 $\boldsymbol{\Omega^{-1}cm^2mol^{-1}}$ which indicates that it is a non-electrolyte which lies within octahedral geometry in DMF as reported [1] and [6].

The electronic spectra of both the ligands and their complex are presented in Table 4. The free ligand of cloxacillin exhibits three bands at 202 nm, 207 nm, and 239 nm while the free ligand of cephalexin exhibits two bands of 220 nm and 233 nm respectively. These were assigned in the assignment of $\boldsymbol{n-\pi^*}$, $\boldsymbol{\pi-\pi^*}$, CT- band, $\boldsymbol{n-\pi^*}$ $\boldsymbol{and}$ $\boldsymbol{\pi-\pi^*}$. These absorption bands also appeared in the electronic spectra of the complex, but they are shifted to different values which proves the coordination of the ligands to the central metallic ions [11]. Ni (II) complex shows three bands at the regions (221 nm, 43478 cm^{-1}), (230 nm, 43478 cm^{-1}) and (237 nm, 42194 cm^{-1}) which are attributed to metal to ligand charge transfer (MLCT), $^3A_2g \rightarrow {}^3T_2g$, and $^3A_2g \rightarrow {}^3T_1g$ transitions for an octahedral Ni (II) complex [10].

Table 5: C.H.N.S. Elemental Analysis and Metal Content of the Synthesized Complex

Compounds	Molar Mass (g/mol)	Elemental and metal analysis found (calculated) %				
		C	H	N	S	M
Ni(Cep)(Clox)Cl_2]	$C_{35}H_{35}N_6S_2O_9Ni$ (805.69)	46.07 (52.13)	3.68 (4.34)	8.96 (10.43)	5.52 (7.9)	8.00 (7.28)

The result of the elemental analysis of the Ni (II) complex is presented in Table 5. From the result obtained, the percentage of C, H, N, and S are in conformity with the proposed structure. Based on the evaluation of the elemental analysis data, it shows that the compound analysed as [Ni((Cep)(Clox)Cl_2)] which shows the coordination of the metal to the two ligands as 1:1:1. The percentage of the metal ion also agree with the proposed structure. The result is in line with that of [7] and [14].

Table 6: Antibacterial test of the Antibiotics and their Metal (II) Complex

Compounds	Concentration ($\boldsymbol{\mu g}$)	Staph. Aureus (mm)	Salmonella typhi (mm)	E. Coli (mm)
Cloxacillin	4 000	11	10	10
	2 000	10	11	11
	1 000	12	8	8
	500	9	7	7
Cephalexin	4 000	11	11	10
	2 000	9	11	11
	1 000	7	9	9
	500	00	8	7
[Ni(Cep)(Clox)Cl_2]	4 000	16	15	17
	2 000	15	14	15
	1 000	14	15	13
	500	8	00	7
Standard				
Ciprofloxacin	500	30	27	25
	200	-	-	-

From Table 6, the result of the antibacterial test for the cloxacillin and cephalexin as well as their metal (II) complex are presented. The result shows that the free ligands as well as the mixed complex were active against all the three isolates of bacteria in all concentrations especially at higher ones. However, at lowest concentration of 500 $\boldsymbol{\mu g}$| agar-well, inactivity was observed by cephalexin ligand against staphylococcus aureus. Likewise, the complex exhibited inactivity against Salmonella typhi isolate. It can be observed that the nickel (II) complex of the mixed antibiotics has shown a better antibacterial activity than the individual antibiotics (ligands). Similar result was reported by [6].

Based on the analytical data obtained such as melting point, UV-spectroscopy, elemental analysis, magnetic susceptibility, and FTIR spectral studies, the tentative proposed structure of the metal (II) complex of the mixed antibiotics can be illustrated as follow:

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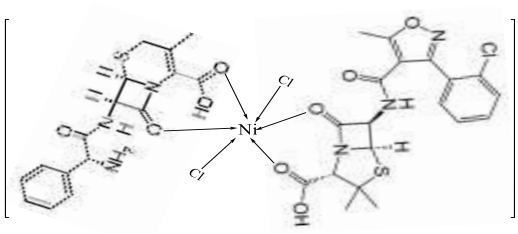

Proposed Structure of the [Ni (clox)(cep) Cl_2]

CONCLUSION

Cloxacillin and cephalexin are among the interesting antibiotics which are used as ligands in synthesizing metallic complexes to improve their activity by some researchers using refluxing solution-based synthesis. This work has demonstrated the use of such method to obtain similar result with effective antibacterial activity.

From the values obtained in conductivity, magnetic measurement, and spectral studies, octahedral geometry is suggested. In the spectral studies of both IR and UV-Visible spectroscopy, the ligands (antibiotics) are coordinated to the metal ion to form the complex of [Ni (Cep)(Clox)Cl_2] through the υ (C = O) carboxylic acid and that of amide giving rise to octahedral geometry.

RECOMMENDATION

Since the synthesized complex has been found to be highly active against certain bacteria isolates, this research work therefore recommends that further researches of this nature should be carried out to produce potent antimicrobial drugs.

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