Synthesis, characterization and antibacterial studies of 7-(a-amino-a-phenylacetamido)- 3-methyl-3-cephem-4-carboxylic acid (cephalexine) metal (II) complexes

[1]Musbahu I., [2]Na'aliya J., [3]Ziyaulhaq U. and [4]Hassan D.

[1]Department of Radiological Safety, Nigerian Nuclear Regulatory Authority, North West Zonal Office, Katsina, Katsina State, Nigeria
[2]Department of Pure and Industrial Chemistry, Bayero, University Kano. Nigeria
[3]Sir Usman Nagogo College of Arabic and Islamic Studies, Katsina, Katsina State, Nigeria.
[4]Department of Chemistry, Federal College of Education Katsina, Katsina State, Nigeria
_____

ABSTRACT

Complexes of Co(II), Ni(II), Cu(II), Zn(II), Mn(II) and Sn(II) with an antibacterial drug, 7-(a-amino-a-phenylacetamido)- 3-methyl-3-cephem-4-carboxylic acid (cephalexine) as ligand were prepared and characterized by Infra red spectral analysis, elemental analysis and gravimetric analysis. The IR spectra revealed that the ligand is a bidentate and coordinated to the central metal ions through carboxyl group and β-lactam nitrogen. Elemental analysis showed that all the complexes have octahedral configurations, $[ML_2CL_2]$, where L= Ligand and M= metal. Molar conductance measurement and decomposition temperature analysis indicated that all the complexes are stable non electrolytes chelates respectively. Antibacterial screening of the complexes against some bacterial isolate was also investigated. The complexes were found to have varied degree of inhibitory effect against the bacterial isolates (*Staphylococcus aureus, Escherichia coli, Pseudomanous arigyana, klebsiella pneumonia* and *Salmonella typhy*).

Key words: Antibiotic, Cephalexine, Gravimetric, Chelation and Characterization.
_____

INTRODUCTION

The medicinal uses and application of metals and metal complexes are of increasing clinical and commercial importance [3]. Monograph and major review, as well as dedicated volume testify to the growing importance of the field [13]. The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, ligands as drug which target metal ion in some form, whether free or protein- bound; and secondly, metal-based drugs and imaging agent where the central metal ion is usually the key feature of the mechanism of action[2,8]. Metal ion and metal complexes in clinical use now include diagnostics (radiopharmaceuticals) used for imaging and therapeutic compound (antibiotic, arthritis and chelating therapy). A number of drugs contains metal ion as essential element such as Pt complexes (anticancer drug), Zn complexes (topical antibiotics) and bismuth compound (anti ulcer) and so on [4,5,6,11]. The recognition of the potential employment of metal complexes and chelate in therapeutic applications provide outlet for basic research in bioinorganic chemistry [7]. A lot of studies carried out showed that the biochemical and pharmacological effects of antibiotics when complexed with metal ion become enhanced at site of action [12]. It has been demonstrated through several studies that biologically inactive and less active compounds become active and biologically active compound become more active upon coordination/

_____

chelation with the metal ions. Also chelating properties of antibiotics may be used in metal transport across membranes or attach the antibiotic to a specific site from which it can interfere with the growth of bacteria [15].

β- Lactam antibiotics contain a 4-membered so called β-lactam ring. These are produced by fungi or molds. Those produced by penicillium mold are termed as penicillin and / or likewise, cefalosporins are produced by cefalosporium mold. This class includes natural penicillins, semisynthetic penicillins, cefalosporins, carbapenems and monobactums.The β-lactam antibiotics are known to inhibit the last step in peptidoglycan synthesis by carboxypeptidase and transpeptidase enzymes, the cross-linking of peptide side chains. They normally act as bactericidals[1]. This paper reported the synthesis of cephalexine complexes of some divalent metals and determined their effect on some bacterial isolates.

Schematic diagram of 7-(a-amino-aphenylacetamido)- 3-methyl-3-cephem-4-carboxylic acid, (Cephalexine) is presented below:
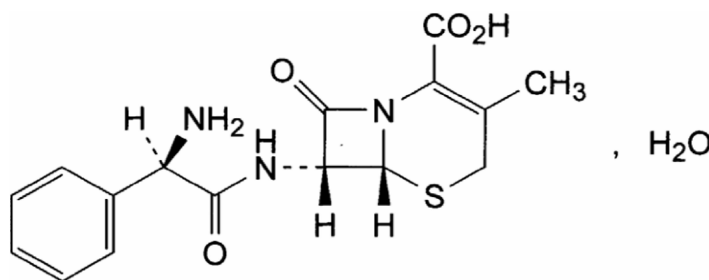

Fig 1: CEPHALEXINE MONOHYDRATE

MATERIALS AND METHODS

MATERIALS
All glass wares used were washed and rinsed with HNO_3 and distilled water, dried in an oven at $110^{\circ}C$ before used. All weightings were carried out on an electric mettler balance. Decomposition temperatures were determined using WRS-IB microprocessor melting point apparatus. Molar conductance measurement was carried out in DMF using jenway conductivity meters DDS- 307 model. Elemental analysis for C, H and N were carried out on flash 2000 Organic Analyzer at The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Infrared spectra of the ligand and its metal complexes were recorded using Shimadzu FTIR-84005 Fourier Transform Infrared Spectrometer in KBr in the range $400\text{-}4000cm^{-1}$, at Multi User Laboratory, Ahmadu Bello University, Zaria. Biological activity studies were carried out at the microbiology unit of the Department of Biological Science, Bayero University, Kano, Nigeria.

REAGENTS
All reagents used in this work were of Analer grade. Cephalexine was obtained from Sigma pharmaceutical company and was used without further purification. Sodium salt of Cephalexine was prepared by reacting equimoler amount of sodium hydroxide with cephalexine. All metal salts were used as chlorides.

EXPERIMENTAL PROCEUDURE
Preparation of Cephalexine Metal Complexes
An ethanolic solution (20ml) of the metal (II) chloride salts (0.001mole) was added to a magnetically stirred sodium salt of cephalexine (0.002mole) in distilled water (25ml). The mixture was refluxed for 1 hr and cooled to room temperature. On cooling, colored precipitate were formed, filtered and washed with ethanol, acetone and ether, and dried by suction. The complexes were re-crystallized in an aqueous ethanol (70:30) gave the desired metal complexes.

METAL ANALYSIS OF THE COMPLEXES
Digestion Method
0.2g of each of the complexes was placed in a beaker containing $25cm^3$ of distilled water. $5cm^3$ of concentrated nitric acid was added followed by a vigorous stirring with a glass rod. The mixture was heated on a hot plate to

_____

about dryness, which was cooled to room temperature. The sides of the beaker was well washed with $25cm^3$ distilled water and filtered. This is termed as digested solution.

DETERMINATION OF CHLORINE

The percentage of chlorine in the complexes was determined by digestion method. 0.2g of the complexes was dissolved in 250ml distilled water with a constant stirring. 0.5ml of concentrated nitric acid was added. To the cold solution 0.1M silver nitrate was added and heated to about boiling. White precipitate was formed on cooling, then filtered and weighed.

MOLAR CONDUCTIVITY MEASUREMENT

0.1g of cephalexine metal (II) complexes was dissolved in 10ml Dimethylformamide (DMF). The conductivity of the resulting solution and that of the 10ml DMF was taken using Jenway conductivity meters DDS- 307 model [16].

Molar Conductivity = 1000K/C

Where C= Concentration
K=Specific conductivity

ANTIMICROBIAL ACTIVITY TEST

The ligand and complexes were screened for their activity against clinically isolated Gramm negative bacteria, *Escherica coli*, *pseudomanous arijina*, *klebsiella pneumonia*, *Salmonella typhy* and gramm positive bacteria, *staphylococcus aureus*. The ligand and the complexes were dissolved separately in dimethylformamide (DMF) to produced three different concentrations (15μg, 30μg and 60μg) per disc. The prepared disc of the ligand and the complexes together with the standard antibiotics disc were placed onto the surface of the inoculated media at an interval and incubated at $37^{\circ}C$ for 24hrs [6,9]. The in vitro antibacterial activity was carried out by the disc diffusion method. The diameter of the zones of inhibitions produced by the ligand and complexes were compared with the standard antibiotics.

RESULTS AND DISCUSSION

Table 1: Physical and Analytical data of the metal (II) complexes

Metal(II) complexes	Color	Melting Point($^{\circ}$C)	Decomposition temperature($^{\circ}$C)	Molar Conductance (ohm^{-1}cm^{2}mol^{-1})	Elemental Analysis Calculated/(Found) %				
					C	H	N	M	Cl
Cephalexine		178							
[NiL$_2$Cl$_2$]	Brown		222	0.52	46.51 (46.49)	4.41 (4.39)	10.17 (10.30)	7.10 (7.34)	8.58 (7.70)
[MnL$_2$Cl$_2$]	Brown		225	2.49	46.72 (46.80)	4.41 (4.46)	10.22 (10.31)	6.68 (6.66)	8.62 (7.60)
[CoL$_2$Cl$_2$]	Black		224	0.30	46.49 (46.41)	4.39 (4.37)	10.71 (10.31)	7.13 (6.99)	8.58 (8.20)
[CuL$_2$Cl$_2$]	Green		221	0.58	46.24 (46.46)	4.37 (4.39)	10.11 (10.23)	7.64 (7.57)	7.46 (8.00)
[ZnL$_2$Cl$_2$]	Orange		225	0.87	46.13 (46.29)	4.36 (4.42)	10.01 (10.17)	7.85 (7.20)	8.51 (7.45)
[SnL$_2$Cl$_2$]	Pale white		220	0.44	47.58 (47.64)	4.49 (4.53)	10.40 (10.51)	14.40 (13.90)	8.78 (8.20)

The interaction of the antibiotic (ligand) and the divalent metals resulted in a dark colored complexes of different colors, this is due to the absorption of a photon of light, resulting in a splitting of degenerated orbital in the metal ions and subsequent transition of electrons in the metal complexes. The ligand is soluble in water and in most organic solvents while all the complexes are insoluble in water and in all other common organic solvents except DMSO and DMF.

The molar electrical conductance of the complexes in DMF at 25°C are in the range of 0.30-2.50 ohm^{-1}cm^{2}mol^{-1} which is far less than that of KCl used for standardization, indicated that there is no mobile ions in the complexes and they are non-electrolyte in solution[16]. The high values of decomposition temperature obtained indicated that all the complexes are stable. (Table 1)

_____

Table 2: Infra-red Spectral Analysis of the Metal(II) Complexes

Complexes	v(N-H)cm^{-1}	v(C=O)cm^{-1}	v(C-O)cm^{-1}	v(M-N)cm^{-1}	v(M-O)cm^{-1}	v(M-Cl)cm^{-1}
Cephalexine	3390	1760	1066			
[CoL$_2$Cl$_2$]	3430	1623	1085	461	845	631
[NiL$_2$Cl$_2$]	3420	1628	1031	408	910	639
[CuL$_2$Cl$_2$]	3444	1632	1093	431	832	632
[ZnL$_2$Cl$_2$]	3410	1629	1073	421	922	630
[MnL$_2$Cl$_2$]	3315	1652	1055	457	930	640
[SnL$_2$Cl$_2$]	3409	1635	1080	420	941	628

The IR spectrum of 7-(a-amino-a-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid (cephalexine) showed some characteristics bands at 3390cm^{-1}, 1763cm^{-1} and 1066cm^{-1} assigned to v(N-H$_2$), v(C=O) and v(C-O) vibrations, respectively. The metal complexes contained all the bands observed in a ligand and also other bands, an indicative of the coordination of the ligand with the metal ions. The vibration assigned to $\bar{v}$(N-H$_2$) at 3390cm^{-1} in the ligand shifted to a lower frequency in the spectra of all the complexes. And a new band at 405-465cm^{-1} assigned to $\bar{v}$(M-N) was evolved in the spectra of the complexes indicating the involvement of NH$_2$ via nitrogen in the coordination. Also the vibration at 1066cm^{-1} in the spectrum of the ligand assigned to v(COO) shifted to a higher frequency in all the complexes an indicative of a complexation [10]. A new absorption band instead, assigned to v(COO) and v(M-O) appeared at (1031-1094)cm^{-1} and (850-1010)cm^{-1} respectively, this in turn indicated that carboxyl group is coordinated to the metal ions [14]. More over a strong bond at (550-650) cm^{-1} is also observed in the spectra of all the complexes which is assigned to $\bar{v}$(M-Cl) [12], Table 2.

Table 3 : Antimicrobial Activity

Test Organisms	Compound	Zones of inhibitions(mm)/conc.(µg)			Control(mm)
		60	30	15	
Staphyloccocus aureus	Cephalexine	12	10	08	Pefloxacine(30mm)
	[MnL$_2$Cl$_2$]	12	09	06	
	[CuL$_2$Cl$_2$]	13	10	08	
	[CoL$_2$Cl$_2$]	10	07	06	
	[SnL$_2$Cl$_2$]	11	09	06	
	[ZnL$_2$Cl$_2$]	13	11	08	
	[NiL$_2$Cl$_2$]	10	08	06	
Escherichia coli	Cephalexine	11	10	06	Septrin (35mm)
	[MnL$_2$Cl$_2$]	14	12	09	
	[CuL$_2$Cl$_2$]	12	10	07	
	[CoL$_2$CL$_2$]	11	08	07	
	[SnL$_2$Cl$_2$]	10	08	07	
	[ZnL$_2$Cl$_2$]	13	11	08	
	[NiL$_2$Cl$_2$]	11	09	07	
Pseudomanous arigyana	Cephalexine	11	10	07	Gentamycine(12mm)
	[MnL$_2$Cl$_2$]	12	09	08	
	[CuL$_2$Cl$_2$]	10	08	06	
	[CoL$_2$Cl$_2$]	06	06	06	
	[SnL$_2$Cl$_2$]	12	07	06	
	[ZnL$_2$Cl$_2$]	12	09	07	
	[NiL$_2$Cl$_2$]	10	08	07	
Klebsiella pneumonia	Cephalexine	10	07	06	Ampicilline(28mm)
	[MnL$_2$Cl$_2$]	10	07	07	
	[CuL$_2$Cl$_2$]	13	10	08	
	[CoL$_2$Cl$_2$]	11	08	07	
	[SnL$_2$Cl$_2$]	06	06	06	
	[ZnL$_2$Cl$_2$]	06	06	06	
	[NiL$_2$Cl$_2$]	12	09	08	
Salmonella typhy	Cephalexine	12	08	07	
	[MnL$_2$Cl$_2$]	13	10	09	
	[CuL$_2$Cl$_2$]	11	08	06	
	[CoL$_2$Cl$_2$]	10	07	06	
	[SnL$_2$Cl$_2$]	10	09	07	
	[ZnL$_2$Cl$_2$]	11	09	06	
	[NiL$_2$Cl$_2$]	06	06	06	

_____

The C, H, N elemental analysis and metal percentage composition of all the complexes, indicated that the metal - ligand ratio is 1:2, $[ML_2X_2]$ where M= Co, Ni, Mn, Zn, Cu and Sn. L= 7-(a-amino-a-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid (Cephalexine), X= Cl.

The antibacterial activity test of 7-(a-amino-a-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid (ligand) and its metal (II) complexes have been determined. The diameter of zones of inhibition (mm) was measured for each treatment. The ligand showed minimal activity against all test organisms at all concentrations. Even though, 7-(a-amino-a-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid is not active against *Escherichia coli* and *klebsiella pneumonia* at low concentration   15μgml⁻¹, but all the metal (II) complexes showed activity against *escharica coli* and *klebsiella pneumonia* at all concentrations, with little improvement in Mn(II), Cu(II), Zn(II), Co(II), and Ni(II) metal complexes. *Klebsiella Pneumonia* was resistant to Sn(II) and Zn(II) metal complexes. Cu(II) and Zn(II) showed moderate increase of activity against *staphyloccocus aureus*, while Mn(II), Co(II), Sn(II), and Ni(II) metal complexes were not active at low concentrations. Mn(II) is the only complex that showed increase activity against *salmonella typhi* and *pseudomanous ariyana*

CONCLUSION

Complexes of 7-(a-amino-aphenylacetamido)-3-methyl-3-cephem-4-carboxylic acid (ligand) with metal(II) chloride were synthesized and studied by various analytical procedures including solubility test, conductance measurement and decomposition temperature, they were further characterized by elemental analysis, infrared spectral analysis and gravimetric analysis. The ligand and the metal complexes were also screened against bacterial extracts. The complexes were found to be octahedral, non-ionic, stable chelates with enhanced antibacterial activity when compared to the parent drug.

REFERENCES

[1] A Tomasz; **1986**, Review of Infectious Diseases. 8, supplementary 3, 260-278

[2] A.G. Brown and S.M. Robert; **1984**. Recent advances in chemistry of beta lactams Antibiotics. *The royal society of Chemistry,* London.

[3] B.S Singh; **2011**,"inorganic/bioinorganic: biological, medicinal and pharmaceutical uses". *Journal of pharmaceutical educational research,* vol 2

[4] D.R. Williams, **1971** "The Metals of Life" Van Nostrand, London.

[5] E.G. Sanders, L. D. Wright and D. B. McCormick; **1965.** *journal of Biological. Chemistry*, 240, 362 *inorganic chemistry"* wiley.

[6]J Sunil, P Vatsala, and V. Uma; **2011**, *Reserch journal of pharmaceutical, Biology And chemical science"* Vol. 2:61

[7] J.A Obaleye, J.B. Nde-aga, E. A Balogun; **1997** *African Journal of Science*. 1: 10,12.

[8] K.H Thompson; **2010** "Medicinal Inorganic Chemistry: An introduction. *Encyckopedia of*

[9] M Zaworotko, J Hammud, H Hassan, A Ismail, K.C Victor, M Masoud and S mamdoud; **2006**, *Journal of coordination chemistry* 59(1): 65-84

[10] M. Z Yongxing, M Z Yun and Z. Gang;**1989**, *Inorganic Chimistrym* Acta, 165, 185

[11] M.J. Seven and L. A. Johnson, "Metal Binding in Medicine", Lippincott Co, Philede

[12] N Sultana, N, Arayne, M Saeed: **2007**, *parkistan journal of pharmaceutical science,* , 20(4) :305-310

[13]N.P Farrel; **1999**, "The uses of inorganic chemistry in medicine" *the royal society ofchemistry:*Cambridge

[14] P. A Ketchun; **1988**, "Microbiology: concepts and applications," John Wiley and Sons, N.Y, p. 495-496.

[15] R, Juran Anacona,, T Caredemy; **2004**. *Journal of transitional meatl chemistry* 26(1.2):228-231

[16] W. j Geary; **1971**, *Coordination chemistry Review* 7(1):82-110