Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(4):588-593

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, characterization and antimicrobial studies of metal (II) complexes of ciprofloxacin

Mustapha A. N.[1], Ndahi N. P.[2], Paul B. B.[3] and Fugu M. B.[2]

[1]*Department of Chemistry, Federal College of Education Yola, Adamawa State, Nigeria*
[2]*Department of Chemistry, University of Maiduguri, Borno State, Nigeria*
[3]*Department of Vet. Surgery and Theriogenology, University of Maiduguri, Maiduguri, Borno State, Nigeria*

ABSTRACT

Metal complexes of ciprofloxacin with Co(II), Ni(II) and Cu(II) chloride salts were prepared using a literature procedure. The complexes were characterized by conductivity measurement, Uv/Visible and FTIR spectroscopy. Based on the IR, Uv/visible spectroscopies and microanalysis data, the compounds formed by the ciprofloxacin have their formula proposed as $[M(Cp)_2Cl_2).6H_2O]$- 1 where M= Ni and $H_3Cp[MCl_2(H_2O)_2]$- 2 where M= Co or Cu. The ligand and the complexes showed varying degrees of solubilities in polar and non-polar solvents. Conductivity measurement showed that all the complexes were non electrolytes (14.4 – 49.9 S cm^2 mol^{-1}). IR and microanalysis data revealed that ciprofloxacin acted as a bidentate ligand which coordinated to the metal ions through the ring carbonyl oxygen and one of the O atom of the carboxylate group. The complexes were also tested for their antimicrobial activity against eight different bacterial strains such as Esherichia coli, Klebsialla pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis and Corynebacterium pneumoniae at 20mg/ml, 15mg/ml and 10mg/ml concentration levels using agar disc diffusion method. The results showed that all the ciprofloxacin complexes were highly efficacious against all the tested strains except Staphylococcus aureus.

Key words: Metal complexes, ciprofloxacin, antimicrobial, antibiotics, ligand

---

INTRODUCTION

The emergence and spread of pathogens resistant to many available drugs is of great concern. The situation is critical in Africa as a result of the spread of resistance to the inexpensive drugs widely used for treatment of diseases such as malaria and tuberculosis. As an alternative, a number of metallic ion-drug combinations are being assayed and suitable ones recommended. However the question about cost and adequacy of the supply necessitate the need to identify new novel agents [1]. What are needed to fight those multi-resistant bacteria are antibiotics with novel activity. Metal complexes in particular offer great promise for such novel activity. The synthesis of metal-drugs complexes have been known to broaden the spectrum of their activity including antibiotics. Such activity was exhibited by mixed ligand metal complexes of ampicillin, and chloramphenicol prepared by using Ni(II), Co(II), and Fe(III) metal chloride hexahydrate [2]. Many compounds have been prepared based on well conceived ideas of improving their efficacy and have been subsequently screened with few, successfully passing clinical trials [3]. Ciprofloxacin (quinolone) has been suggested to be such novel agents that can be complexed to metallic ions to enhance their efficacy. Quinolone antibiotics are complexing agents for a variety of metal ions including alkaline earth metals.

The antimicrobial activity of ciprofloxacin complexes against the multi-resistant organisms is very scanty. This study therefore presents the synthesis, characterization and antimicrobial activity Co(II), Ni(II) and Cu(II) complexes of ciprofloxacin ligand.

EXPERIMENTAL SECTION

The metal(II) salts were obtained in the form of their chlorides and were of analaR grade purchased from BDH and they were used without further purification. They are: cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate and copper(II) chloride dihydrate while The ligand: ciprofloxacin was obtained from Fidson Healthcare Nigeria drugs. The bacterial strains used are four Gram- negative bacterial strains (*Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and four Gram-positive bacteria (*Bacillus subtilis, Corynebacterium pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes*) which were obtained from the University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Preparation of the Complexes

The complexes were prepared using hydrated metal(II) chlorides of cobalt, nickel and copper with ciprofloxacin drugs in mole ratio 1:2, (M : L) [4, 5, 6]. The drugs were carefully grounded manually into fine powder, using pestle and mortar. The general equations are presented as follow:

$MCl_2.\ 6H_2O + 2Cp \rightarrow [M(Cp)_2Cl_2).6H_2O] + 2HCl$ where M = Ni, Cp = ciprofloxacin

$MCl_2.nH_2O + 2Cp \rightarrow H_3Cp[MCl_2(H_2O)_2]$ where M = Co or Cu.

Preparation of Ciprofloxacin-Cobalt(II) Complex

The complex was prepared by using the method adopted by Obaleye *et al*., (2007) and Arayne *et al*., (2009) by dissolving 10mmole (3.31g) ciprofloxacin in 20ml hot methanol. Five millimoles (5mmole), (1.189 g) cobalt(II) chloride hexahydrate dissolved in 10ml hot methanol was added with constant stirring and refluxed for 2hours. The mixture was then transferred to a beaker and left in a refrigerator for 30minutes. The brown precipitate was washed with (3 x 5)ml portions distilled water and dried in dessicator over anhydrous calcium chloride for three days.

Preparation of Ciprofloxacin-Ni(II) Complex

The complex was prepared by dissolving 20mmole (6.62g) of finely powdered ciprofloxacin in 20ml hot methanol. Ten millimole (2.377g) of nickel((II) chloride hexahydrate was dissolved in 10ml hot methanol. The two solutions above were mixed and refluxed for 2 hours with constant stirring. The mixture was then carefully poured into a beaker and cooled to room temperature before filteration. The precipitate was washed with (3 x 5ml) portions methanol and also with distilled water. The precipitate was dried using similar procedure already described.

Preparation of Ciprofloxacin-Copper(II) Complex

Ten millimole (3.31g) ciprofloxacin drug was dissolved in 20ml hot methanol and mixed with 5mmole (0.853g) copper(II) chloride dihydrate dissolved in 10ml hot methanol. The mixture was refluxed with constant stirring for 2 hours. The content was then transferred to a beaker and cooled to room temperature. After filtration, the green precipitate was washed with 3 - 5ml petroleum ether followed by distilled water. The precipitate was dried as described above.

Physical measurements

The percentage metal ions in the complexes were determined by EDTA complexometric titration [7]. Microanalysis was carried out with Perkin Elmer model 2400 series II CHNS/O elemental analyzer in the Department of Chemistry, University of Zululand, South Africa.

Melting points and decomposition temperatures of the ligand and the metal(II) complexes were determined on Griffin melting points apparatus. The results are presented in Table 1.

The solubilities of the complexes were determined in different solvents ranging from polar to non-polar such as distilled water, methanol, ethanol, ethylacetate, chloroform, acetone, petroleum ether and benzene. The results are presented in Table 2.

Molar conductivity for the complexes were recorded using Exstic ® series model EC 500 conductivity/temperature meter at the Department of Soil Science, University of Maiduguri, Nigeria. Concentration of 10^{-3} molar solutions of the complexes were prepared in methanol and acetone. The results are presented in Table 1.

Infrared spectra

The Fourier transform infrared (FT-IR) spectra were recorded on Shimadzu FTIR 8400S model spectrophotometer at National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria, in the range 4000 - 500cm^{-1} as KBr pellets. Spectra were recorded for both the ligands and the metal(II) complexes. The data are presented in Table 3.

Electronic Spectra

The electronic absorption spectra of the complexes were recorded on UV/visible spectrophotometer (UV-2500PC series) at National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria, at wavelength range of 250 - 600 nm. The samples were dissolved in dimethylsulphoxide (DMSO). The results are presented in Table 4.

Antimicrobial Activity Test

Antimicrobial test was carried out in the Department of Veterinary microbiology, Faculty of Veterinary medicine, University of Maiduguri. Antimicrobial activities of the parent drug (ligand) and the metal(II) complexes were tested against eight different species of bacteria (Gram-positive and Gram-negative) namely: *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aureus, Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium pneumoniae* and *Bacillus subtilis.* The bacterial isolates were collected from University of Maiduguri Teaching Hospital, Maiduguri, Borno State. Nigeria. Nutrient agar was used as the bacteriological growth media. The bacterial activities in the presence of both the parent drug and the complexes were determined by filter paper disc agar diffusion method [8].

Bacterial Isolates

Twenty eight grams of nutrient agar was dissolved in 1litre of distilled water (2.8%) and allowed to soak for 10 minutes, swirled to mix and then sterilized in autoclave at 121^{O}C at 15lbs for 21 minutes. The mixture was cooled to 47^{o}C and mixed well before pouring 20ml each into sterilized plates. The pH of the medium was 7.3 ± 0.2. The agar was allowed to cool and solidify.

The inoculated discs containing various concentrations (20mg/ml, 15mg/ml and 10mg/ml) of the parent drugs and the complexes were then arranged and pressed firmly to the inoculated agar surface with sterile forceps. The impregnated discs were sufficiently spaced out to prevent overlapping of the zones about 15mm from the curved sides of the plates and 40mm from an adjacent disc. A pre-diffusion time of 30 minutes was allowed and all the plates were incubated at 37^{o}C for 24 hours after which they were observed and recorded. The zones were interpreted using scoring method following interpretative chart of Kirby- Bauer sensitivity method [8].

RESULTS AND DISCUSSION

The Co(II) and Ni(II) inorganic complexes of ciprofloxacin exhibited higher melting points than parent drugs, this is similar to complexes reported by Ogunniran *et al.*, (2008). The molar conductivities of all the complexes ranged from 14.4 to 49.9 S cm^2 mol^{-1}, indicating that they are non electrolytes. The complexes are blue, green or white. The blue or green colours are typical of transition metals because of d –d transition. The percentage yields of the complexes are in the range 49 – 63 %.

The microanalysis data of the prepared complexes as presented in Table 2. The results showed that the ciprofloxacin complexes analysed into two types as: $[Ni(Cp)_2Cl_2].6H_2O$ –1 and $H_3Cp[MCl_2(H_2O)_2]$ – 2. Where M= Co and Cu.

IR spectra

The assignments of the bands are based on similar studies of drug-based metal complexes [4, 9, 13]. Most of the spectra that are present in the free ligand also appeared in their metal complexes due to their structural similarity. The appearance of bands at 765 and 940 cm^{-1} in Co(II) complex of ciprofloxacin which could not be traced to the free ligand spectrum is attributed to M-O bond. The M-O bands appeared at 756 cm^{-1} and 752 cm^{-1} for Ni(II) and Cu(II) complexes respectively. The bands due to M-Cl were not found because of instrument limitation. Microanalysis results, however, agrees with the proposed structures. The v(O-H) band at 3529 cm^{-1} in ciprofloxacin

shifted to 3530 and 3413 cm^{-1} in Co(II) and Cu(II) complexes respectively. The disappearance of the band at 1474 cm^{-1} in ciprofloxacin due to (COOH) group suggests that the coordination was through O atom of the carboxylic group. The appearance of new bands at 765w, 758w and 752w cm^{-1} were assigned to the M-O in Co(II), Ni(II) and Cu(II) complexes respectively sequel to chelation [5].

In Co(II) and Cu(II) the carboxylic group is not deprotonated; therefore the ciprofloxacin is not directly coordinated to the metal. It is proposed for this class of compounds, the ligand exist as cation. The positive charge on ciprofloxacin ion is neutralized by the dichlorometate anion. Similar ionic complexes have been reported by other researchers [4, 11, 12]. The octahedral geometry assumed by Ni(II) complex agrees with the Uv/visible spectrum and similar to the structures of Ni(II) complexes reported by Ogunniran *et al*., (2008) and Arayne *et al*., (2009) [2, 4, 5, 9].

Electronic spectra

Ciprofloxacin-Co(II) complex showed two close peaks in the Uv region with λmax at 315.8nm (31666cm-1). This may be assigned to $\pi - \pi^*$ transition of the chromophores. While the weak band at 30248 cm^{-1} may be assigned to charge-transfer transition [14,15]. Ni(ii) complex showed well defined single band in the UV region with λmax at 366.4nm (27,293). This is also due to $\pi - \pi^*$ transition of the chromophores.

Antimicrobial studies

The complexes were tested against different strains of bacteria at concentrations of 20 mg/ml, 15mg/ml and 10mg/ml. The Co(II) complex showed higher activities against all the tested strains (*E.coli, Salmonella typhi, Klebsiella, Pseudomonas, Streptococcus pyogenes, Corynebacterium pneumoniae* and *Bacillus subtili*s) similar to the parent drug [4] except against *Staphylococcus aureus.* Large inhibition zones (21- 40 mm) was shown by Ni(II) complex for all the test strains except against *staphylococcus aureus*. Similar activities were observed against *Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Bacillus subtilis*. Increased activity was shown against *Escherichia coli* and *Corynebacterium pneumoniae* compared to the parent drug. In Cu(II) complex no activity was found against *Staphylococcus aureus*, but increased activity was observed against *E. coli, Salmonella typhi, Streptococcus pyogene, Klebsiella pneumoniae, Pseudomonas aeruginosa, Corynebacterium pneumoniae* and *Bacillus subtillis* compared to the parent drug.

Table 1: The Physical Properties of the Ligand and the Complexes

Compound	Colour	Yield(g) %	M. pt/d (°C)	Λm (S cm^2mol^{-1})
Ciprofloxacin	White		255-257	
Cipro-Co(II)	Blue	2.96 (49)	300-306d	14.4
Cipro-Cu(II)	Green	2.38 (63)	220-222d	21.8
Cipro-Ni(II)	White	2.60 (56)	300-304d	49.9

d = decomposition temperature, M. pt = melting point, S = Siemen and Λm = molar conductivity.

Table 2: Microanalysis of Co(II), Ni(II) and Cu(II) complexes

Compound	Mol. Formula/mol. Mass	Microanalysis: found (calculated) %			
		C	H	N	M
$H_3Cp[CoCl_2(H_2O)2$	$C_{17}H_{25}O_5N_3FCl_2Co$ (500.33)	41.14 (40.81)	5.68 (5.04)	8.05 (8.40)	11.79 (11.77)
$H_3Cp[CuCl_2(H_2O)2$	$C_{17}H_{25}O_5N_3FCl_2Cu$ (509.90)	40.44 (40.44)	5.01 (4.99)	5.87 (8.40)	12.65 (12.57)
$Ni(Cp)_2Cl_2.6H_2O$	$C_{34}H_{48}O_{12}N_6F_2Cl_2Ni$ (900.67)	45.63 (45.35)	5.70 (5.37)	6.49 (9.33	6.29 (6.52)

Table 3: Relevant IR Frequencies and electronic spectra (cm^{-1})

	V(O-H)	(N-H)	v(NH2)	v(COO)	v(C=O)	v(C-O)	v(C-N)	v(M-O)	λmax (cm^{-1})
Cipro	3529s, 3377b	3092w	3093w	1396w 1477s	1622s	1323m	1269s		
Cp-Cu(II)	3413b	--	3091w	1390w 1478s	1622s	1305m	1264w	752w	23365
Cp-Ni(II)	3529s, 3381b	3092w	3091w	1392w	1623s	1332w	1269s	758w	27293
Cp-Co(II)	3530s	3381b	3092w	1395w 1460s	1623s	1325m	1268s	765w	31666 30248

S = sharp, b = broad, m = medium, w = weak

Table 4. Antimicrobial Activities of Ciprofloxacin and its Metal(II) Complexes (Zone of inhibition in mm)

Microorganism	Concentration (mg/ml)											
	Ciprofloxacin			Cp-Co(II)			Cp-Ni(II)			Cp-Cu(II)		
	20	15	10	20	15	10	20	15	10	20	15	10
Bacillus subtilis	36	34	30	25	23	21	30	28	24	30	28	10
Cory. pneumoniae	35	33	31	27	24	21	40	38	35	30	27	22
Escherichia coli	35	32	30	28	26	21	38	36	34	33	31	30
Klebs. pneumoniae	37	34	30	30	27	25	35	32	30	35	33	25
Pseud. aeruginosa	36	32	30	29	22	19	27	24	21	28	26	20
Salmonella typhi	46	43	39	30	28	25	36	34	18	35	32	18
Staphyl. Aureus	32	30	28	0	0	0	0	0	0	0	0	0
Strept. Pyogenes	40	37	34	21	19	17	30	28	27	30	28	28

CONCLUSION

In this paper metal (II) complexes of ciprofloxacin drug were synthesized and characterized. The complexes were generally stable and sparingly soluble in most of the solvents used. The study revealed that they are non electrolytes. The Ni(II) complex showed octahedral geometry. The ligand coordinated to the metal ions through the ring carbonyl oxygen atom and oxygen atom of the carboxylate group. The antimicrobial activity of the complexes revealed a reduced activity against the test strains compared to the parent drug. The structures of the complexes are proposed thus:

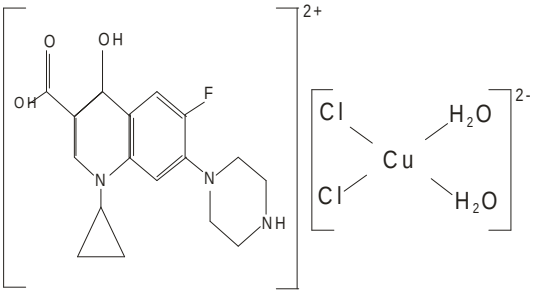

Fig. 1: Cu(II)- ciprofloxacin complex

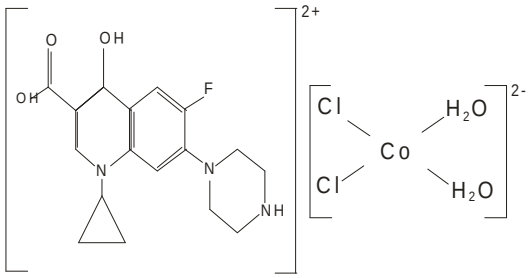

Fig. 2: $H_3Cp[CoCl_2(H_2O)_2]$

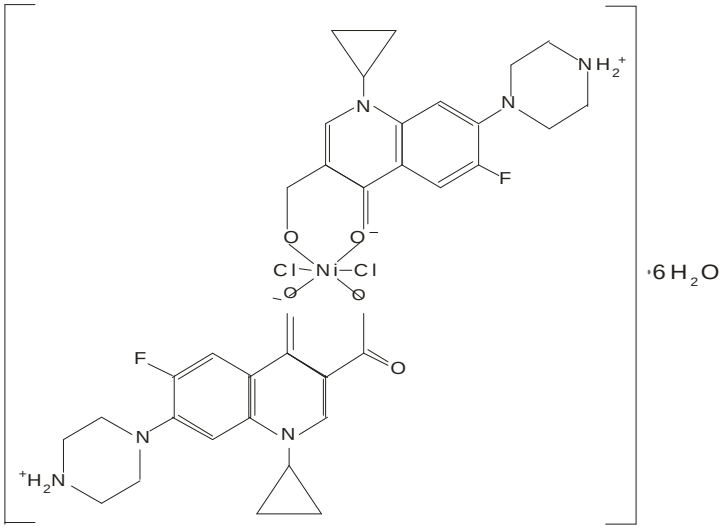

Fig. 3: Ni(II)- ciprofloxacin complex

Acknowledgements

The authors wish to acknowledge Professor G. A Kolawole of Department of Chemistry, University of Zululand South Africa for his technical assistance. We are also grateful to College of Education Waka-Biu, Borno state, Nigeria for the financial grant.

REFERENCES

[1]GG Mohammed; M.M Omar and A.M Hindy. *Turkey J. Chem.* **2006**, 30 (3): 361-382
[2]KO Ogunniran; KO Ajanaku; OO James; JA Adekoya and OC Nwinyi. *African J. Pure and Applied Chemistry.* **2008,** 2 (7): 69-74.
[3]JD Paul. and S. Giann. *Dalton Transition Res*. **2006**, 10: 1929-1933.
[4] JA Obaleye and A Lawal. *J. Applied Science, Environmental Management.* **2007**, 11 (4): 15-18.
[5]S Arayne; N Sultana; U Haroon; and M.M Ahmed. *Bioinorg. Chem. and Appl.* **2009**, 20: 321-327
[6] MB Fugu; NP Ndahi; BB Paul and AN Mustapha. *J. Chem. and Pharm Res.* **2013**, 5(4): 22 - 28
[7] AI Vogel. *A Textbook on Practical Organic Chemistry including Qualitative Organic Analysis* (4th Edition). Longman group Ltd London: **1978**, 264 - 269
[8] M Cheesebrough. *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press. **2004,** .223 - 229.
[9] KO Ogunniran; AC Tella; M Alensela and MT Yakubu. *African J. Biotechnology*. **2007**, 6(10): 1202 -1208
[10] AA Osunlaja; NP Ndahi and JA Ameh. *African J. Biotechnology.* **2009**, 8(1): 4-11
[11] M Zupancic; I Turel; P Bukovec; AJ White and DJ William. *Croatica Chem.. Acta.* **2001,** 74(1): 61-74
[12] I. Turel; K. Gruber; I. Leban and N. Bukavec. *J. Inorgan. Biochem.* **1996**, 61: 197- 212.
[13] EV Sekhar: KN Jayaveera and S Srihari. *J. Chem. and Pharm. Res.* **2012**, 4(12): 5121 - 5125
[14]MM El-ajaily; AA Maihub; SS Hudere and SM Bensaber. *Science and its Application Journal*. **2005**, 1: 196 - 210.
[15]S Kumar and S Rahang. *J. Chem. and Pharm. Res.* **2013**, 5(1): 45 - 48