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Physiochemical and Biological Study on Ciprofloxacin Complex

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Abstract: The antibacterial drug 2,2 -bi pyridyl, 1,1- cyloprop -yl-6, u-oxo-1,4-dihydroquiline-3- carboxylic and piperazinyl (ciprofloxine) and its copper complex have been qualitatively and quantitatively analyzed by electrochemical, IR, microbial and pharmacological method.

Ciprofloxine gives well defined polarographic reduction wave/peak with $E_{1/2}$ /E_P= -1.42/-1.44V versus SCE in 0.01 M KCl at pH= 8.5 the former wave could be used to determine ciprofloxine between $5x10^{-7}$ and $3x10^{-5}$ M. The method was applied to the determination of ciprofloxine in formulated tables with relatives S.D of less than 0.4 %.

The IR spectral data on the drug and it's Cu(II) Complex revealed complexation through N-atom of azide group. The data showed a shift in the band due to the azid group in the complex from 2270 to $2151Cm^{-1}$ in case of pure drug Ciprofloxine[1]. Hence the tentative structure of the complex has been confirmed Antibacterial activity of the Complex has been determine by inhibitory concentration for the bacteria strain which is measured by the agar incorporation method in plastic petridishes against. E.coli, Salmonella typhi. Vibrochloerae. and Diplococcus Pneumonia bacteria.
Keywords: Anti bacterial activity, Ciprofloxine, Cu(II) complex, Polarography.

Introduction

The survey of relevant literature reveals that Cu (II) have been proven beneficial against several disease such as tuberculosis, gastric ulcer and cancers[2]. The coordination Chemistry of these drugs with metals ions of biological and pharmaceutical[3-6] importance is of considerable interest ciprofloxacin was considered the best of the third generation quinolone family. Recently we have found the synthesis of a copper complex containing the fluoroquinolone ligand, the Ciprofloxine found was characterized by FT-IR, elemental analysis. The crystal structure of this copper complex was also characterized and is reported in this paper.

Experimental Procedure

All the chemical were used of analytical grade and which has used without further purification sigma laboratory, USA, Supplied the ciprofloxine drug. Ciprofloxine (0.01M) and KCL (0.1M) have been prepared in conductivity water.

1. **Polarograpy** All the Polarography measurement have been, recorded on an Elico (India) Pulse Polarograpy model CL- 357 comprising of three electrodes system a dropping mercury electrode (DME) as working electrode a saturated calomel electrode (SCE) as reference electrode and a coiled platinum wire as an auxiliary electrode.

   Experimental sets were prepared by keeping over all Cu (II) potassium chloride supporting electrolyte, concentration fixed at 1.0mM and 1.0M respectively. The pH of the test solution was adjusted to 6.0 $\pm$ 0.1 as using NaOH/HCl solution Hydrogen gas was bubbled through the test solution for about 15min before recording the polarogram. The ligand (Ciprofloxacin) concentration was varied from 0.01mM to 5mM.

2. **Amperometric titration of Copper (II) – ciprofloxine complex -**

   For amperometric titration sets of solution and containing varying concentration of drug were prepared in 0.1M potassium chloride and titrated against the standard solution of the titled Cu(II) ions whose pH has been adjusted to that of the titrate(7.0±0.1). The platue potential for Cu (II) is -0.3V vs SCE is fixed at potentiometer.

Synthesis of solid complex

A cream colored complex has synthesized by refluxing aqueous solution of Cu (II) and ciprofloxine on 2:1 molar ratio for about 3h. The complex has marked by precipitation after reducing the volume of reaction mixture to one fourth of the volume the product has filtered, washed, dried over P_4O_{10} and characterized.

IR - spectrometric measurement

The IR spectrophotometric analysis in solution phase using Perking Elemer-379, IR spectrophotometer was done.

Antimicrobial Screening

Raper's method was followed for microbial screening of the complex against various pathogenic bacteria Viz E-Coli, Salmonella typhi (A. aniger & A. Flavus) number of replicates in each of the case was three. The percentage inhibition was calculated using the formula.

$$\% \text{ inhibition } \frac{a-b}{a} \times 100$$

Where a, = diameter of inhibition Zone for complex
b = diameter of inhibition Zone for metal.

Results & discussion

In 0.01M KCl at pH 6.0 $\pm$ 0.1 ciprofloxine produced a well-defined polarographic signal in and DCP and DPP model with half-wave potential $E_{1/2}$ = -1.65V vs SCE and peak potential EP=1.42V verse SCE id/ip has found to be proportional to the ciprofloxine concentration under the above mention conditions, ciprofloxine is sold under market as antibacterial (100 mg CaPS) medicine,which is soluble in distilled water. Method of standard addition was used to determine the ciprofloxine content of antibacterial powder, it manufacture "Burrough wellcome", India, thus enabling the quality control of ciprofloxine in pharmaceutical formulation.

In 0.1M KCl at pH = 6.0 $\pm$ 0.1, Cu (II) and its complex with ciprofloxine produced a well defined reversible and diffusion controlled Polarographic wave which has revealed by the log plot slope and the plots of i_d versus $\sqrt{h}$ effective hight of mercury column respectively on the gradual addition of the ligand the $E_{1/2}$ of the metal shifted to more electronegative values indicating the formulation of Cu(II) - ciprofloxine complex Lingane's treatment of the observed polarographic data revealed 1:1 [Cu(II)- ciprofloxine] complex formulation is solution with $\log \beta_1 = 4.48.\sqrt{h}$

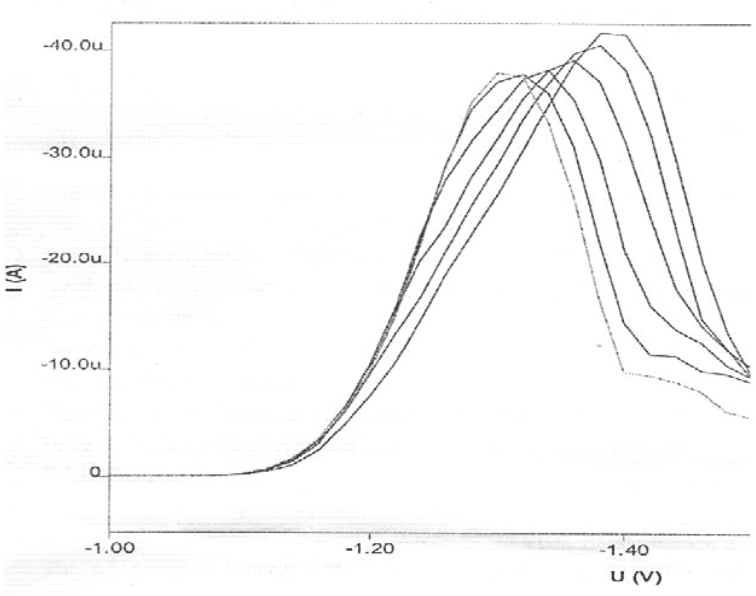


Fig 1 Polarograms of Fe (III) (1mM) in 0.1M KCl supporting electrolyte at pH 7.0±0.2 and
1. Without drug, 2. 1mM drug, 3. 2mM and 3mM Ciprofloxacin

Amperometric titration of ciprofloxacin With Cu (II) ion

On gradual addition of Cu(II) ion the ciprofloxine analyzed solution, the current goes on decreasing to minimum and then attends a constant value the plot of $i_d\sqrt{h}$ (v+v/v) versus volume of titrant added to the titre, revealed a L-shaped curve the end point has indicate by the intersection of the two line which confirmed 1:1 ML complex formation.(Fig 2)

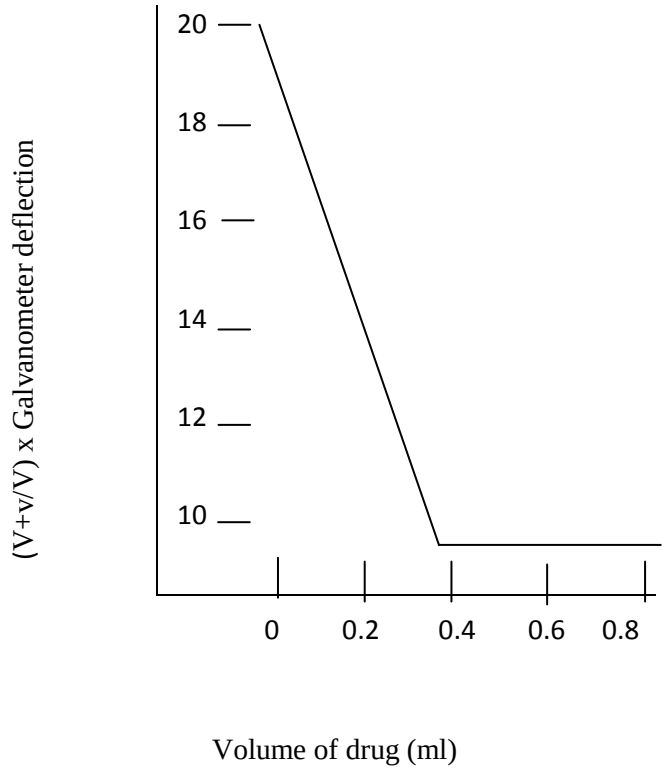


Fig-2 Amperometric titration of (2mM/10ml) Ciprofloxacin
(1mM/10ml) Cu(II) solution

The IR- Spectra of quinolones

The analytical data of the ligand and its complexes along with some physical properties are summarized in Table(1). The ligand on interaction with Cu(II), chloride yield complexes corresponding to the general formula (ML_2). The low molar conductance value of the complexes reveals their non-electrolytic nature[7]. These complex are more soluble in-organic solvents and less soluble in inorganic solvent. Elemental analysis data (Table) show that the metal to ligand ratio is 1:2.

In order to study the binding mode of both the ligand to the metal in complexes the IR spectra of the uncompleted ligand were compared with the spectra of metal complexes. The IR spectra of the ligands show a band in the region 1725-1730cm^{-1} and 1248-1254 cm^{-1} assignable to the COOH group[8] the absence of these band in metal complexes.

Reveals the deprotonation of the COOH group on complexation. These ligands also exhibit their characteristics band in the region 1650-1630 cm^{-1} assigned azomethine linkage in the ligand. The IR spectra of metal complexes indicate shifting of these band to lower frequencies (1610-1590 cm^{-1}).

Thus confirming the involvement of the azomethine group in bonding with metal ion. The above description of IR data supported the mono anionic bi dentate nature of these investigated ligands. The IR spectra of metal complexes also show new band in the region 470-455 cm^{-1} and 390-350 cm^{-1}.

Antimicrobial activity*

The minimal Inhibitory concentration for the bacteria strain listed in table 2 has measured by the agar incorporation method in plastic petri-dish containing muceller Hinton hinton agar (100ml) and incorporating the antimicrobials in concentration of 0.014 to 63 µg / ML in doubling dilution, following the method of the National centre for drug screening[9,11].

Synthesis of Metal complex with ciprofoxine

To a solution of ciprofloxine (2m mol) in methanol [(10ml), a solution of Metal chloride or sulphate] salt (2m / mol) in methanol (10ml) was added, the mixture has stirred at heated or refluxed at 60^0C for 1hr. The resulting solution was filtered. Precipitate formed for Cu (II) Ciprofloxine Complex the filtrate has evaporated to one third of the initial volume and refrigerator for 2 week this result into formation of crystals; re-crystallization of the crystals has carried out to give suitable crystal for analysis.

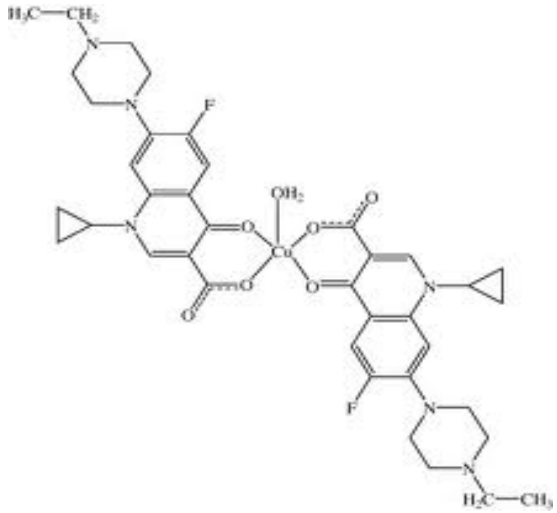
Table 1: IR data of ligand of metal – complexes

Compound	**IR (cm^{-1})**						
	(COO^-) v (C-o)	(COO^-) vc= (C-o)	V_a (COO^-)	Vs (COO^-)	V (C=N)	V (M-N)	V (M-O)
Drug	1726	1253	-	-	1626	-	-
$Cu(cif)_2H_20$	–	-	1631	1483	1609	459	379

Table – 2

Compound				
	Staphylococcus A	Micrococcus, Lukens	Fcoli	Pseudomonas & argue in
Ciprofloxine	<20	<20	>20	<20
Cu(cif) $_2$.3 H_2o	<20	>20	<20	>20

Reaction



Antimicrobial Screening

Mice for experiment have been injected as describe by San chez - Delgado (1969). Swiss mice have divided into 43-group and 3 in each group, Kept in metal Cage and fed with mice cuber and water alibitum.

Group A: Control
Group B: Ciprofloxine
Group C: $Cu(CPf)_{2.}3H_2o$

Formula -

$$\% \text{ inhibition} = \frac{100 - \text{estimated number parasites treated mice}}{\text{Estimated number of parasites in un treated mice}}$$

Treatment of animals

Male albino rats (wistar strain), weighing 160-180g have obtained from the zoology department of chemical science, Ajayi Gowther, university oyo, Nigeria, they have been kept in wire meshed cages and fed with commercial rat chow (Bendal feed Nigeria Ltd.) and supply water alibitum[12,13].

12 rats have divided randomly into 3-group Group A [control] received distill water, Group B have been administer with free ligand [ciprofloxine], Group C have been administrated with $Cu(CPf)_{2.}3\ H_2o$.

The distilled water and solution of ligand and metal complex have been administered orally to the rate in the various group three time daily for 15 day, 30 days 45 days, 60 day at a dose of 3.33 mg/kg body weight.

Collection of blood sample for Serum Preparation

The rats have been sacrificed by stunning and cervical dislocation. Blood sample has collected into clean labeled sample bottles. The clear liquid on top has centrifuged at 3000mg for 10 min and the supernatant [Serum] has used for the enzyme assay.

Preparation of tissue homogenates

The method described by yakubu 2005 has used to prepare the tissues. The liver and kidney have been quickly excised from the rats blotted of strain and homogenized in 4 volume of iced cold 0.25m sucrose the homogenates have been kept in well- labeled containers and Store in the freezer for further use.

Determination of serum and tissue

ALP Activities

The serum and tissues ALP activities have been determined using the randox diagnostic kit laboratories manual [1997]. ALP activity determination has based on the method of Wright et. al 1972 modified by akanji 1989[14].

Protein determination

Protein determination in the serum and the tissue homogenates has estimated by the method of lowry (1951).

Statistical analysis

The data have been analyzed using one way ANOVA, followed by Duncan multivariable 'post hoc' test for comparison between control and treated rats in all group P>0.05 have been considered Statistically significant.

The effects of oral administration of the ligands and the complexes on rat liver, kidney and Serum alkaline phosphate activities are shown in table (4). The serum ALP activities showed slight Significant change [P>0.05] on comparison with one another and the control after repeated administration of ligand and complexes. The fact that there has no significant difference in serum ALP activities of ligand and ligand-Metal complexes administered rats when compared with control suggested that the integrity of the plasma membrane was not compromised.

Moreover, the observed significant increases in the ALP activities of the liver and kidney of rate administered with ciprofloxine Complexes suggests and enhancement of the activities of the enzyme by the drugs and their metabolites. The increase may also be as result of stress imposed on the tissue by the drugs, which may lead to loss of the enzyme molecule through leakage into extra cellular fluid which has not been significantly noticed in the serum. In a bid to offset this stress, the tissue may increase the de novo synthesis of the enzyme thus accounting for increase in ALP activities in these tissues[15].

The research show that the antibacterial activity of ciprofloxine lignad is more potent as compare to the pure drug. The percentage, of reductions are bacteria 67%, 76%, for ciprofloxine respectively.

Table - 3

Compound	**Milting point ($_0$C)**	**Colour**	**% yield**	**% metal Content theoretical [experiment]**	**Conductivity (2^{-1}) cm^{-1} dm^{-3}**
Ciprofloxine	192-193	Cremey	70.30	6.13 (6.25)	9.7×10^{-7}
Cu(cip) $_2$3. H_2o	205-206	Blue	75.30	6.13 (6.25)	1.17×10^{-5}

Table - 4

Ligand / complexes	**Kidney IV / L**	**Liver IV / L**	**Serum IV / L**
Control	62.17 $\pm$ 4.10	21.03 $\pm$ 2.80	11.7 $\pm$ 0.95
Ciprofloxine	73.30 $\pm$ 5.08	6.13 $\pm$ 3.14	11.00 $\pm$ 0.77
Cu(cip) $_2$3 $H_2$0	75.70 $\pm$ 5.10	27.30 $\pm$ 1.06	8.24 $\pm$ 0.64

Table – 5: Percentage parasitemia of metal complexes

Complex	**Number**	**Average % parasitaemia before administration**	**Average of % parasitaemia after administration**	**% reduction parasitaemia**
Cu(cip) $_2$ 3 H_2o	Ci	33	2	91

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