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Synthesis and Spectroscopic Characterization of Zn(II), Cd(II), and Hg(II) Ciprofloxacin Complexes[1]

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Abstract—Ciprofloxacin metal co mplexes with general for mula $[M(CPF)_2]X_2 \cdot nH_2O$ [M = Zn(II), Cd(II), and Hg(II)] have been synthesised and characterized using elemental analysis (CHN), spectroscopic (UV-Vis, IR, MS, and ^{1}H NMR) and ther mogravimetric (TG and DTA) data. Using the Coats–Redfern and Horowitz–Metzeger methods, kinetic analysis of the thermogravimetric data had been performed.

Keywords: ciprofloxacin, thermalanaysis, infrared spectra, kinetic data, complexes

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INTRODUCTION

A number of drugs and potential pharmaceutical agents contain metal-binding or metal-recognition sites, that can bind or interact with metal ions and thus potentially affect their bioactivities. Numerous examples of these "metallodrugs" and "metallopharmaceuticals" and their actions can be found in the literature, for instance: (a) several anti-inflammatory drugs, such as aspirin and its metabolite salicylglycine [1–4], suprofen [5], are known to bind metal ions that affect their antioxidant and anti-inflammatory activities; (b) the potent histamine-H_2-receptor antagonist cimetidine [6] can form complexes with Cu^{2+} and Fe^{3+}, histidine blocker famotidine can also form stable complex with Cu [7, 8], (c) anthelmintic and fungistatic agent thiabendazole, which is used [7, 8] for the treatment of several parasitic diseases, forms a complex with metal:drug ratio of 1 : 2 [9], (d) Ru^{2+} complex of the anti-malarialdrug chloroquine exhibits an activity two to five times higher than the parent drug against drug-resistant strains of Plasmodium faciparum [10].

The term quinolone(s) refers to potent synthetic chemotherapeutic antibacterials [11, 12]. They are highly active against most Gram-negative pathogens including *Pseudomonas aeruginosa* and the *Enterobacteriaceae*. The effectiveness of drugs depends on their binding ability [13–15]. Ciprofloxacin (CPF; Fig. 1) is a second generation fluoroquinone that is bacteriostatic at low concentration and bactericidal at high concentrations.

Almost all of the recent clinically useful quinolones bear a fluorine atom in the C-6 position and thus, these antibacterial agents are called fluoroquinolones. They are effective against Gram-positive and Gram-negative bacteria through inhibition of their NAD gyrase, a critical enzyme to bacterial chromosome replication [16]. Nine coordination compounds of Cu(II) and Co(II) with ciprofloxacin and enoxacin as ligands have been prepared and characterized. The copper ion, at a crystallographic inversion centre, is in a tetragonally distorted octahedral environment [17]. Several Ca(II), Co(II), Ni(II), Cu(II), Zn(II), Al(III), and Fe(III) complexes of the ciprofloxacin were studied by potentiometric and spectroscopic methods in solution, as well as EPR and polarographic methods [18]. A

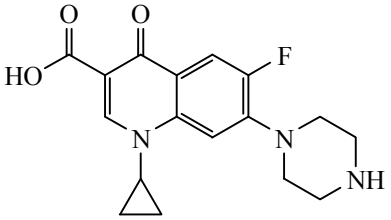

Fig. 1. Scheme of ciprofloxacin.

[1] The text was submitted by the authors in English.

novel mixed-ligand Cu(II) complex of ciprofloxacin and phenanthroline, was found to crystallize as a dimeric moiety containing monocationic and dicationic species [19]. Two such dimeric moieties are found in the same unit cell composing a dicationic cluster. The hydrothermal reaction of ciprofloxacin with $Cu(BF_4)_2 \cdot 6H_2O$ yields a copper complex $Cu(CPF)(BF4)_2) \cdot 6H_2O$, which was characterized by spectroscopic and electrochemical measurements as well as single crystal X-ray studies. Reaction of ciprofloxacin with copper(II) nitrate in the presence of 2,2'-bipyridine resulted in the isolation of the complex $[Cu(CPF)(bipy)(CO_{0.7}NO_3)_{0.31}(NO_3) \cdot 2H_2O$ [20]. Two Fe(III) complexes of ciprofloxacin were synthesized by reaction of the ligand with Fe(III) chloride hexahydrate in different solvents [21]. The nature of bonding of the ligands and the structure of the isolated metal complexes were elucidated on the basis of their physical and spectroscopic studies. The infrared spectra suggest that two classes of compounds were obtained: molecular complex in which the ligands were bidentately bonded to the metal through the ring carbonyl oxygen and one of the oxygen of the carboxylate group and the ionic complex consisting of a tetrachlorometalate ion which is electrostatically attached to the ligand. A vanadium complex [22] was prepared from $VOSO_4$ aqueous solution and ciprofloxacin. The crystals were very unstable and contained high amount of disordered water molecules, so the exact refinement of the structure has not yet been possible. The tentative formula of the complex is $[VO(CPF)_2]SO_4 \cdot 10H_2O$ with a typical chelate bonding of metal to 4-oxo and carboxylic oxygens of quinolone. In the magnesium adduct of CPF $\{(CPF_2)_2[Mg(H_2O)_6](SO_4)_2 \cdot 6H_2O\}$ [23], magnesium is not bonded to the quinolone molecule. The quinolone is protonated at the terminal nitrogen atom of piperazine residue. The hydrogen atom of the carboxylic group is hydrogen bonded to the carbonyl oxygen atom, thus preventing bonding of the metal to this part of the molecule. The magnesium ion is coordinated by six water molecules forming a $[Mg(H_2O)_6]^{2-}$ cation with a nearly regular octahedral geometry. Two Bi(III) compounds $(CPF_3)_2[BiCl_6] \cdot 2H_2O$ and $(CPF_3)_2[Bi_2Cl_{10}] \cdot 4H_2O$ have been prepared [24]. In the former, one of the CPF molecules is protonated at carbonyl oxygen and the terminal nitrogen of the piperazine residue, whereas the other is protonated only at the latter nitrogen atom. The charge of the isolated hexachlorobismuthate(III) anions is compensated by protonated CPF molecules. Due to their high charge, $[BiCl_6]^{3-}$ anions are not very common as the formation of polynuclear anions seems to be preferred [25]. In the latter compound Bi(III) ions are coordinated by chloride ions forming dinuclear $[Bi_2Cl_{10}]^{4-}$ anions. Both quinolone molecules are doubly protonated in this compound.

The synthesis and characterization of new metal complexes with ciprofloxacin are of great importance for understanding the drug-metal ion interaction especially taking into account their potential pharmacological use. The objective of this study was the isolation and characterization of the Zn(II), Cd(II), and Hg(II) complexes, as well as their characterization using spectroscopic and thermal analysis techniques. The thermal behavior of these complexes was also studied.

EXPERIMENTAL

All chemicals used were of the purest laboratory grade (Merck) and ciprofloxacin was a gift from Egyptian international pharmaceutical industrial company (EIPICo.).

Carbon and hydrogen contents were determined using a Perkin-Elmer CHN 2400 instrument. The metal content was found gravimetrically by converting the compounds into corresponding carbides or oxides. The sulfate content in the sulfate containing complexes was determined gravimetrically as barium sulphate using $BaCl_2$ solution as a precipitating agent. Chloride content in all prepared complexes was determined potentiometrically by the titration against a standard $AgNO_3$ solution. Zn(II) ions were determined compleximetrically using EDTA at pH = 10, using ammonia buffer while Cd(II) and Hg(II) were determined by atomic absorption technique. Molar conductivities of freshly prepared 1.0×10^{-3} M DMSO solutions were measured using Jenway 4010 conductivity meter. IR spectra were recorded on a Bruker FTIR spectrometer in 4000–400 cm^{-1} range in KBr pellets. The UV-Vis spectra (1.0×10^{-3} M) were determined in the DMSO using Jenway 6405 spectrophotometer with 1cm quartz cell, in the range 200–800 nm. ^{1}H-NMR spectrum of the free ligands and their complexes were recorded on a Varian Gemini 200 MHz and Bruker Advance 300 MHz spectrometers using DMSO-d_6 as solvent and TMS as an internal reference. Thermogravimetric analyses (TG) was carried out in the temperature range from 25 to 800°C in a steam of nitrogen atmosphere on a Shimadzu TGA 50H machine. The experimental conditions were: platinum crucible, nitrogen

Table 1. Analytical and physical data of CPF and its metal complexes

Complex	M_w	Formula	C, %		H, %		N, %	
			calculated	found	calculated	found	calculated	found
$[Cd(CPF)_2]Cl_2·2H_2O$ (**I**)	871.11	$[Cd(C_{17}H_{18}FN_3O_3)_2]Cl_2.2H_2O$	46.88	46.06	4.63	4.14	9.65	9.23
$[Cd(CPF)_2](NO_3)_2$ (**II**)	899.10	$[Cd(C_{17}H_{18}FN_3O_3)_2](NO_3)_2$	45.42	45.11	4.36	4.13	12.46	12.09
$[Cd(CPF)_2]SO_4$ (**III**)	871.16	$[Cd(C_{17}H_{18}FN_3O_3)_2]SO_4$	46.88	46.23	4.17	4.03	9.65	9.16
$[Hg(CPF)_2]Cl_2$ (**IV**)	934.18	$[Hg(C_{17}H_{18}FN_3O_3)_2]Cl_2$	43.71	43.28	3.88	3.66	8.99	8.77
$[Hg(CPF)_2](NO_3)_2$ (**V**)	987.28	$[Hg(C_{17}H_{18}FN_3O_3)_2](NO_3)_2$	41.36	41.44	3.68	6.18	11.35	11.22
$[Hg(CPF)_2](OAc)_2$ (**VI**)	981.36	$[Hg(C_{17}H_{18}FN_3O_3)_2](OA)_2$	51.40	51.32	4.11	4.33	8.56	8.43
$[Zn(CPF)_2]Cl_2·2H_2O$ (**VII**)	798.98	$[Zn(C_{17}H_{18}FN_3O_3)_2]Cl_2$	51.11	51.02	4.29	4.24	10.51	10.21
$[Zn(CPF)_2](OAc)_2$ (**VIII**)	842.13	$[Zn(C_{17}H_{18}FN_3O_3)_2](OA)_2$	51.10	51.11	5.00	5.11	9.93	9.46

atmosphere with a 30 ml/min flow rate and a heating rate 10°C/min.

Preparation of solid complexes. The ciprofloxacin complexes were prepared using a 1 : 2 (metal ions: CPF) ratio. A solution of 1.0 mmol of a appropriate salt of Zn(II), Cd(II), or Hg(II) previously dissolved in 10 cm^3 of distilled water was added to a solution of 1.0 mmol of ciprofloxacin in 50 cm^3 of acetone. The resulting mixtures were heated at ~60°C under reflux on a water bath for about 10 hours and then cooled. The obtained complexes were separated from the reaction mixture by filtration, washed with boiling water and acetone and dried under vacuum over $CaCl_2$.

MICROBIOLOGICAL INVESTIGATION

The investigated isolates of bacteria were seeded in tubes with nutrient broth (NB). The seeded NB (1 cm^3) was homogenized in the tubes with 9 cm^3 of melted (45°C) nutrient agar (NA). The homogeneous suspensions were poured into Petri dishes. The discs of filter paper (diameter 4 mm) were ranged on the cool medium. After cooling, on the formed solid medium, 2×10^{-2} mL of each of the investigated compounds (1.0×10^{-3} M solution in DMSO) were applied using a micropipette. After incubation for 24 h in a thermostat at 25–27°C, the inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. An inhibition zone diameter over 7 mm indicates that the tested compound is active against the bacteria under investigation [26]. Obtained compounds were tested against *Escherichia Coli* (Gram – ve), *Bacillus subtilis* (Gram + ve) and fungi (tricoderma and penicillium).

RESULTS AND DISCUSSION

The elemental analysis results are summarized in Table 1. These results, as well as the obtained mass spectra are in good agreement with the proposed formula. The melting points of the complexes are higher than those of the free ligand, revealing that the complexes are more stable than ligand. The molar conductance values of the complexes were found to be in the range from 18 to 48 $\Omega^{-1}\,cm^2\,mol^{-1}$ at 25°C, which indicates that the complexes are of a non-electrolytic nature [27]. The low conductivity values are in agreement with the low solubility of CPF complexes in water, ethanol, chloroform, acetone and most organic solvents. On the other hand, they are soluble in DMSO, DMF and concentrated acids.

Infrared spectra. The IR data for ciprofloxacin and its complexes are listed in Table 2. The IR spectra of the complexes were compared with those of the free ligand in order to determine the coordination sites that may be involved in chelation. There are some guide peaks, in the spectra of the ligand, which are useful in achieving this goal. The position and/or the intensities of these peaks are expected to be changed upon chelation. These guide peaks are listed in Table 2. The ν(OH), ν(C=O), ν_{asym}(COO) and ν_{sym}(COO) stretching vibrations are observed at 3530, 1707, 1493, and 1384 cm^{-1} [28, 29] for free ciprofloxacin ligand. The participation of the carboxylate (O) atom in the complexes formation is evidenced from the shift in position of these bands to 3520–3443, 1710–1732 or the disappearance of the bands between 1506–1597 and 1385–1394 cm^{-1} for ciprofloxacin–metal complexes. For comparison the carbonyl-O; ν(C=O),

Table 2. IR spectra (4000–400 cm^{-1}) of CPF and its metal complexes

Compound	n(C=O)	ν(COO) (asym)	ν(COO) (sym.)	ν(C=O) (carbonyl)	ν(M–O) (carbonyl + carboxylate)
CPF	1707 sh	1493 sh	1384 sh	1624 sh	–
I	1725 sh	1506 s	1392 m	1627 sh	496 s, 473 s, 535 s
II	1729 sh	1510 m	1380 s	1628 sh	497 w, 474 w, 536 s
III	1732 m	1550 m	1392 sh	1627 sh	492 s, 474 w, 543 s
IV	1727 sh	1543 m	1383 m	1629 sh	596 w, 471 w, 530 s
V	1720 sh	1541 m	1390 sh	1630 sh	595 s, 469 s, 536 s
VI	1724 sh	1527 m	1392 sh	1628 sh	568 s, 474 w, 532 s
VII	1719 sh	1542 sh	1394 sh	1630 sh	495 s, 470 w, 537 s
VIII	1710 sh	1597 m	1385 sh	1628 sh	496 s, 476 w, 543 s

stretching vibration is found in the free ligand at 1707 cm^{-1} [29]. This band is shifted to higher wavenumbers (1710–1732 cm^{-1}) in the complexes indicating the participation of the carbonyl-O in coordination. New bands are found in the spectra of the complexes in the regions 532–543, 496–568 and 469–476 [28, 29], which are assigned to ν(M–O) stretching vibrations of coordinated water, carboxylate-O and carbonyl-O, respectively. Therefore, from the IR spectra, it is conclude that ciprofloxacin behaves as neutral bidentate ligand and binds to the metal ions through protonated carboxylate O and carbonyl groups.

Electronic absorption spectra. The formation of the M(II) complexes was also confirmed by UV-vis spectra. It can be seen that free ciprofloxacin has two distinct absorption bands. The first one at 285 nm may be attributed to π→π* transition of the heterocyclic moiety and benzene ring. The second band observed at 340 nm is attributed to *n*→π* electronic transition. In the spectra of the M(II) complexes, the two bands are hypochromically affected, obviously suggesting the ligand has changed to the zwitterionic form. The results clearly indicate that the ligand coordinate to metal(II) ions via carboxylic and ketone groups, which is in accordance with the results of the FT-IR spectra.

^{1}H NMR spectra. The ^{1}H NMR spectra further support the assignment of the coordination modes. Upon comparison with the free ligand, the signal observed at 11 ppm can be assigned to the carboxylate –OH. This signal disappears in the spectrum of the Zn(II) complex, which confirms the coordination of CPF ligand to the M(II) ions through the deprotonated carboxylic group. Due to the different chemical environments, two signals are recorded for the quaternized nitrogen ($-^+NH_2$) at δ (2.51 : 2.50) and 2.08 ppm. The peaks at 3.58–3.46 ppm can be assigned to hydration water, which were not detected in the spectrum of the free ciprofloxacin ligand.

Mass spectra. In the mass spectra of Zn(II), Hg(II) and Zn(II) complexes, intense mass peaks at *m/z* = 330, 286, 244, 161, 107, and 56 were detected. The first mass peak corresponds to the $[H–CPF]^+$ ion and the second one proceeds by loss of CO_2 from the molecular ion at *m/z* = 286 with intensity 72%, then the elimination of C_2H_4N leads to the formation of an ion at *m/z* = 244. In comparison between norfloxacin and three ciprofloxacin complexes, the peak assigned to molecular ion *m/z* = 330 of ciprofloxacin ligand is presented in all three complexes, and new peaks appear at *m/z* = 65, 112, and 201 that can be assigned to zinc(II), cadmium(II), and mercury(II) metal, respectively. These results are again consistent with the presence of direct metal-ligand bonding in the three ciprofloxacin complexes.

Thermogravimetric analysis. The weight losses for each chelate were calculated within the corresponding temperature ranges. The different thermodynamic parameters are listed in Table 3.

Kinetic studies. The activation parameters Δ*E*, Δ*H*, Δ*S*, and Δ*G* were obtained from the DTG diagrams using Coats–Redfern [30] and Horowitz–Metzger [31] methods. The activation energies of decomposition were found to be in the range 22.41–265.0 kJ mol^{-1}. The high values of the activation energies reflect the thermal stability of the complexes. The entropy of activation was found to have negative values in all the

Table 3. Thermodynamic data for the thermal decomposition of CPF metal complexes

Complex	Temperature range, °C	E^*, kJ mol^{-1}	A, s^{-1}	ΔS^*, J mol^{-1} K^{-1}	ΔH^*, kJ mol^{-1}	ΔG^*, kJ mol^{-1}	Correlation coefficinet r^2
I	30–140	41.778	2.46×10^5	–142.12	41.23	49.75	0.91
	140–570	33.552	2.17×10^8	–104.94	30.37	63.96	0.91
	570–1000	92.664	6.86×10^7	–135.72	84.88	182.65	0.90
II	300–550	78.826	9.26×10^5	–146.30	75.54	128.26	0.93
	550–900	68.15	1.60×10^8	–115.51	61.37	139.92	0.96
III	30–160	125.29	6.67×10^3	–64.03	124.59	120.43	0.91
	160–520	265.10	2.20×10^3	–136.18	262.24	218.68	0.89
	520–800	111.52	1.69×10^6	–160.56	104.93	211.20	0.92
IV	50–150	151.06	4.42×10^3	–108.55	150.28	142.61	0.99
	150–420	65.34	1.33×10^5	–161.48	62.87	106.47	0.89
	420–650	59.784	1.08×10^8	–116.18	54.89	111.82	0.90
V	50–500	22.41	3.03×10^9	–87.63	18.42	50.84	0.90
VI	30-100	61.96	1.16×10^8	–85.86	61.41	66.57	0.93
	100–280	74.27	9.56×10^5	–153.36	72.47	100.07	0.98
	280–500	88.18	3.35×10^5	–184.86	85.90	145.60	0.91
VII	30–140	51.43	1.23×10^6	–128.15	50.83	59.16	0.90
	200–310	136.32	5.06×10^8	–96.47	133.20	164.04	0.92
	450–620	85.68	5.80×10^7	–136.50	78.66	167.31	0.92
VIII	40–150	123.97	6.03×10^3	–113.96	122.98	111.65	0.93
	150–550	57.372	1.44×10^7	–133.68	53.57	104.39	0.95
	550–1000	91.17	8.13×10^7	–133.90	83.38	179.79	0.91

complexes, which indicate that the decomposition reactions proceed with a lower rate than the normal ones. In another words, the thermal decomposition process of all ciprofloxacin complexes are non-spontaneous, i.e, the complexes are thermally stable. The correlation coefficients of the Arhenius plots of the thermal decomposition steps were found to lie in the 0.90 to 0.99 range, showing a good fit with linear function.

Microbiological investigation. Antibacterial and antifungal activities of the ciprofloxacin ligand and its complexes were carried out against the Escherichia Coli (Gram – ve), Bacillus subtilis (Gram + ve) and antifungal (tricoderma and penicillium activities.) The antimicrobial activity estimated based on the size of inhibition zone around dishes. The complexes were found to have high activity against *Bacillus subtilis* and *Penicillium*, wher.eas Hg(II) complex is more active than the Zn(II) and Cd(II) complexes against *Tricoderma*.

Structure of the ciprofloxacin complexes. The structures of the complexes of ciprofloxacin with Cd(II), Hg(II) and Zn(II) ions have been confirmed from the elemental analyses, IR, molar conductance,

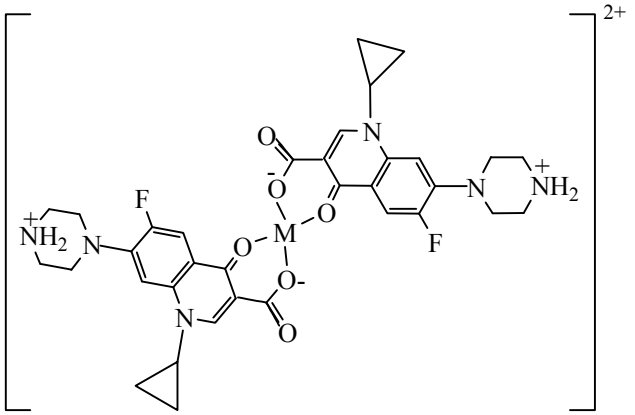

Fig. 2. Structure of the ciprofloxacin complexes.

UV-Vis, mass and thermal analysis data. Based on the above observations, tetradentate geometries was suggested for the investigated complexes (Fig. 2).

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