Bull. Chem. Soc. Ethiop. **2015**, 29(2), 259-274.
Printed in Ethiopia
DOI: http://dx.doi.org/10.4314/bcse.v29i2.9
ISSN 1011-3924

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME NEW TRANSITION METAL COMPLEXES WITH CIPROFLOXACIN-IMINE

S.A. Sadeek[1*], M.S. El-Attar[1,2] and S.M. Abd El-Hamid[3]

[1]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt
[2]Medical Chemistry Dept., Preparatory Year Deanship, Jazan University, Saudi Arabia
[3]Drinking water and sanitation company, Mansoura, Egypt

(Received November 25, 2014; revised May 26, 2015)

ABSTRACT. Some new transition metal complexes of ciprofloxacin-imine derived from ciprofloxacin and o-phenylenediamine were synthesized and characterized on the basis of melting point, magnetic moment, conductance measurements, elemental analysis, infrared, UV/Vis, nuclear magnetic resonance spectroscopy, mass spectra as well as thermal analyses. The data indicate that the ligand acts as tetradentate chelate bound to the metal ions through the deprotonated carboxylate and the azomethine group. The ligand as well as their metal complexes was also evaluated for their antibacterial activity against several bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and also, fungicidal activity aganist *Candida albicans* and *Aspergillus fumigatus* were tested. It was found that the metal complexes are more antibacterial as compared to uncomplexes ligand and no antifungal activity observed for ligand and their complexes. Also, this study showed that the ciprofloxacin-imine is more antibacterial as compared to ciprofloxacin alone.

KEY WORDS: Shiff base, Metal complexes, TG, IR, Mass spectra

INTRODUCTION

Schiff bases are some of the most widely used organic compounds. They are used as pigments, dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers [1]. Schiff bases have also been shown to exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties [1, 2] and also used in the design and development of anticancer chemotherapeutic agents [3]. Imine or azomethine groups are present in various natural, natural-derived, and non natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities [4-6].

Schiff base ligands are able to coordinate to many different metals and stabilized in various oxidation states [7]. The Schiff bases complexes have been used in catalytic reactions and as models for biological systems [8, 9]. Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry [10, 11]. Also aromatic Schiff bases or their metal complexes catalyze reactions on oxygenation, hydrolysis, electro-reduction and decomposition [12-14]. Earlier work has shown that some drugs studied increased activity when administrated as metal chelates rather than as organic compounds [15, 16] and that the coordination possibility of o-phenylenediamine has been improved by condensation with a variety of carbonyl compounds. Tetradentate Schiff bases with a N_2O_2 donor atom set are well known to coordinate with various metal ions, and this has attracted many authors [17, 18].

Some Co(II), Cu(II) and Fe(II) Schiff base chelate complexes show catalytic activity in oxygenation of alkene and increase the rate of hydrolysis more than simple salt [10, 13]. Some metal complexes of a polymer bound Schiff base show catalytic activity on decomposition of hydrogen peroxide and oxidation of ascorbic acid [14]. The work reported herein is focused on the synthesis, spectroscopic and characterization of four new metal complexes $[Cr(CIP\text{-}o\text{-}phdn)(H_2O)_2](CH_3COO)_3.10H_2O$, $[Mn(CIP\text{-}o\text{-}phdn)(H_2O)_2]SO_4.10H_2O$, [Fe(CIP-o-

*Corresponding author. E-mail: s_sadeek@zu.edu.eg

phdn)$(H_2O)_2$]$(NO_3)_3.8H_2O$ and [Co(CIP-o-phdn)$(H_2O)_2$]$Cl_2.7H_2O$ formed upon the reaction of $Cr(CH_3COO)_3$, $MnSO_4.6H_2O$, $Fe(NO_3)_3.9H_2O$ and $CoCl_2.6H_2O$ with new ciprofloxacin-imine (CIP-o-phdn) in the presence of acetone as a solvent and also, to study the effect of condensation of o-phenylenediamine with ciprofloxacin to produce new compound CIP-o-phdn on the antibacterial activity compared with free ciprofloxacin. The solid reaction products were characterized by melting point, molar conductivities, magnetic properties, elemental analyses, infrared spectra, H^1 NMR, mass spectra, UV spectra as well as thermal analyses. The antibacterial activity of the investigated complexes, metal salts and CIP-o-phdn was tested against two Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacterial species *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal screening was studied against two species *Candida albicans* and *Aspergillus fumigatus*.

EXPERIMENTAL

Chemicals

All chemicals used for the preparation of the complexes were of analytical reagent grade, commercially available from different sources and used without further purification. Ciprofloxacin hydrochloride used in this study was purchased from the Egyptian International Pharmaceutical Industrial Company (EIPICO). o-Phenylenediamine, glacial acetic acid, acetone, ethanol, NaOH, $FeCl_3.6H_2O$, $BaCl_2$, $AgNO_3$, $FeSO_4$ and K_2CrO_4 were purchased from Fluka Chemical Co. $CoCl_2.6H_2O$, $Cr(CH_3COO)_3$, $MnSO_4.6H_2O$ and $Fe(NO_3)_3.9H_2O$ from Aldrich Chemical Co.

Synthesis of ligand CIP-o-phdn ($C_{40}H_{42}N_8O_4F_2Cl_2$)

An ethanolic solution of ciprofloxacin (2 mmol, 0.734 g) with o-phenylenediamine (1 mmol, 0.108 g) was boiled under reflux in the presence of glacial acetic acid separately for 4 h. The resulting solution was concentrated to 8 mL on a water bath and allowed to cool at 0 ^oC. Yellowish white preciptate was filtered off, washed several times by ethanol and dried under vacuum over $CaCl_2$ in a disecator. The proposed formula of the ligand ($C_{40}H_{42}N_8O_4F_2Cl_2$, M.Wt. = 807) is in good agreement with mass spectrum (M^+) at m/z = 806.0 (68.91%) and confirmed by IR spectral data. The ^1H NMR spectrum of the ligand in DMSO-d_6 showed signals at δ 11.0 ppm assigned to the proton of carboxylic (COOH).

Synthesis of metal complexes

The grey solid complex [Cr(CIP-o-phdn)$(H_2O)_2$]$(CH_3COO)_3.10H_2O$ was prepared by adding 0.5 mmol (0.164 g) of chromium acetate $Cr(CH_3COO)_3$ in 20 mL ethanol drop-wisely to a stirred suspended solution 0.5 mmol (0.403 g) of CIP-o-phdn and 1 mmol (0.04 g) NaOH in 50 mL ethanol. The reaction mixture was stirred for 15 h at 35 ^oC in water bath. The grey precipitate was filtered off and dried under vacuum over anhydrous $CaCl_2$. The yellow, orange and green solid complexes of [Mn(CIP-o-phdn)$(H_2O)_2$]$SO_4.10H_2O$, [Fe(CIP-o-phdn)$(H_2O)_2$]$(NO_3)_3.8H_2O$ and [Co(CIP-o-phdn)$(H_2O)_2$]$Cl_2.7H_2O$ were prepared in a similar manner described above by using acetone as a solvent and using $MnSO_4.6H_2O$, $Fe(NO_3)_3.9H_2O$ and $CoCl_2.6H_2O$, respectively, in 1:1 molar ratio. All compounds were characterized by their elemental analysis, molar conductance, magnetic moment, infrared, ^1H NMR, electronic, mass spectra as well as thermal analyses. Single X-ray diffraction measurements could not be obtained due to the formation of non suitable crystals.

Elemental C, H and N analysis was carried out on a Perkin Elmer CHN 2400. The percentage of the metal ions were determined gravimetrically by transforming the solid products into metal oxide or sulphate, and also determined by using atomic absorption method. Spectrometer model PYE-UNICAM SP 1900 fitted with the corresponding lamp was used for this purposed. The chloride content in the complexes was determined by using Mohr's and Volhard's methods [19]. IR spectra were recorded on FTIR 460 PLUS (KBr discs) in the range from 4000-400 cm^{-1}, ^1H NMR spectra were recorded on Varian Mercury VX-300 NMR Spectrometer using DMSO-d_6 as solvent. TGA-DTG measurements were carried out under N_2 atmosphere within the temperature range from room temperature to 800 ^oC using TGA-50H Shimadzu, the mass of sample was accurately weighted out in an aluminum crucible. Electronic spectra were obtained using UV-3101PC Shimadzu. The solid reflection spectra were recorded with KBr pellets. Mass spectra were recorded on GCMS-QP-1000EX Shimadzu (ESI-70 eV) in the range from 0-1090. Magnetic measurements were carried out on a Sherwood scientific magnetic balance using Gouy method using $Hg[Co(SCN)_4]$ as calibrant. Molar conductivities of the solution of the ligand and metal complexes in DMF at 1×10^{-3} M were measured on CONSORT K410. All measurements were carried out at ambient temperature with freshly prepared solution.

Antimicrobial investigation

Antibacterial activity of the ligand and its metal complexes was investigated by a previously reported modified method of Beecher and Wong [20] against different bacterial species, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal screening was studied against two species, *Candida albicans* and *Aspergillus fumigatus*. The tested microorganisms isolates were isolated from Egyptian soil and identified according to the standard mycological and bacteriological keys for identification of fungi and bacteria as stock cultures in the microbiology laboratory, Faculty of Science, Zagazig University. The nutrient agar medium for antibacterial was (0.5% peptone, 0.1% beef extract, 0.2% yeast extract, 0.5% NaCl and 1.5% Agar-Agar) and CZAPEKS Dox medium for antifungal (3% sucrose, 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.001% $FeSO_4$, 2% Agar-Agar) was prepared [21] and then cooled to 47 ^oC and seeded with tested microorganisms. Sterile water agar layer was poured then solidified. After solidification 5 mm diameter holes were punched by a sterile cork-borer. The investigated compounds, ligand, metal salts and their complexes, were introduced in Petri-dishes (only 0.1 mL) after dissolving in DMF at 1.0×10^{-3} M. These culture plates were then incubated at 37 ^oC for 20 h for bacteria and for seven days at 30 ^oC for fungi. The activity was determined by measuring the diameter of the inhibition zone (in mm). Growth inhibition was calculated with reference to the positive control, i.e., (ampicilin, amoxycillin and cefaloxin).

RESULTS AND DISCUSSION

The complexes of CIP-o-phdn with Cr(III), Mn(II), Fe(III) and Co(II) were synthesized as solids of a color characteristics of the metal ion. Table 1 summarizes the carbon, hydrogen, nitrogen, halogen, sulfur and metal percentages, melting points, molar conductivities and magnetic properties of the isolated solid complexes. The results obtained indicated that all of the isolated complexes are formed from the reaction of the metal salt with CIP-o-phdn in 1:1 molar ratio for all the metals. All of the complexes reported here in are hydrates with various degrees of hydration and air stable solids at room temperature. The structures of the complexes suggested from the elemental analysis agree quite well with their proposed formulae. The found values of quantitative analysis agree quite well with the calculated percentage of C, H, N, Cl and S. The

metal content is in a well agreement with each other and proves the molecular formulae of the prepared complexes. The molar conductance values of CIP-o-phdn and their metal complexes were found to be in the range from 113.1 to 235.8 S. cm^2. mol^{-1} at room temperature (Table 1). Conductance data showed that the metal complexes are electrolyte compared with ciprofloxacin Schiff base alone. The magnetic moments (as B.M.) of the complexes were measured at room temperature. The Cr(III), Mn(II), Fe(III) and Co(II) complexes are found in paramagnetism with measured magnetic moment values at 3.81, 5.62, 5.81 and 5.10 B.M., respectively. The biological activities of Schiff base and their metal chelates were studied against some selected Gram-positive and Gram-negative bacteria and two species fungi.

For the isolated complexes of Cr(III), Mn(II), Fe(III) and Co(II) in order to verify that the acetate, sulfate, nitrate and chloride groups are ionic and not coordinate, the complexes solution were tested with an aqueous solutions of ferric chloride, barium chloride, ferrous sulfate and silver nitrate a red brown, white precipitate, black-ring and white precipitate were formed. This indicate that acetate, sulfate, nitrate and chloride groups are found as counter ions (outside the complexes sphere) [22] which also in good agreement with the results of molar conductance.

Table 1. Elemental analysis and physico-analytical data for CIP-o-phdn and its metal complexes.

Compounds M.Wt. (M.F.)	Yield %	Mp °C	Color	Found (calcd.) (%)						μ_{eff} (B.M.)	Λ S cm^2 mol^{-1}
				C	H	N	M	Cl	S		
(CIP-o-phdn) 807 ($C_{40}H_{42}N_8O_4F_2Cl_2$)	80.0	308	Yellowish white	(59.48) 59.47	(5.20) 5.17	(13.88) 13.87	-	(8.80) 8.79	-	Diamagnetic	113.1
[Cr(CIP-o-phdn)(H_2O)$_2$](CH_3COO)$_3$ $10H_2O$ 1179 ($CrC_{46}H_{73}N_8O_{22}F_2$)	68.45	270	Grey	(46.82) 46.80	(6.19) 6.15	(9.50) 9.46	(4.41) 4.41	-	-	3.81	196.0
[Mn(CIP-o-phdn)(H_2O)$_2$]SO_4.$10H_2O$ 1101 ($MnC_{40}H_{64}N_8O_{20}F_2S$)	73.30	294	Yellow	(43.50) 43.46	(5.81) 5.75	(10.17) 10.15	(4.99) 4.92	-	(2.92) 2.90	5.62	190.0
[Fe(CIP-o-phdn)(H_2O)$_2$](NO_3)$_3$.$8H_2O$ 1155.8 ($FeC_{40}H_{60}N_{11}O_{23}F_2$)	69.82	>360	Orange	(41.53) 41.50	(5.19) 5.15	(13.32) 13.29	(4.83) 4.80	-	-	5.81	235.8
[Co(CIP-o-phdn)(H_2O)$_2$]Cl_2.$7H_2O$ 1025.9 ($CoC_{40}H_{58}N_8O_{13}F_2Cl_2$)	78.66	282	Green	(46.79) 46.70	(5.65) 5.62	(10.92) 10.89	(5.74) 5.70	(6.92) 6.90	-	5.10	230.8

IR absorption spectra

The infrared spectra of [Cr(CIP-o-phdn)(H_2O)$_2$](CH_3COO)$_3$.$10H_2O$, [Mn(CIP-o-phdn)(H_2O)$_2$]SO_4.$10H_2O$, [Fe(CIP-o-phdn)(H_2O)$_2$](NO_3)$_3$.$8H_2O$, [Co(CIP-o-phdn)(H_2O)$_2$]Cl_2.$7H_2O$ and CIP-o-phdn were measured as KBr discs. The assignments are given in Table 2. The infrared spectra of the complexes are compared with those of CIP-o-phdn in order to determine the site of coordination that may be involved in chelation. There are some guide peaks in the spectra of the ligand which are of good help for achieving this goal. These peaks are expected to be involved in chelation. The position or the intensities of these peaks are expected to be changed upon complexation. The proposed structures for all complexes is represented by Scheme 1, the four donor atoms of CIP-o-phdn coordinated to central metal ions in a plane forming tetragon with the two oxygen atoms of the two coordinated water molecules in axial

position [18]. According to the proposed structures for the complexes under investigation, the complexes posses a two-fold axis and two plane of symmetry and hence they are C_{2v} symmetry. The C_{2v} complexes, $[M(CIP\text{-}o\text{-}phdn)(H_2O)_2]^{n+}$ are expected to display 297 vibrational fundamentals which are all monodegenerate. These are distributed between A_1, A_2, B_1 and B_2 motions; all are IR and Raman active, except for the A_2 modes which are only Raman active.

Table 2. Infrared frequencies (cm^{-1}) and tentative assignments for (A) CIP-o-phdn; (B)$[Cr(CIP\text{-}o\text{-}phdn)(H_2O)_2](CH_3COO)_3.10H_2O$,(C)$[Mn(CIP\text{-}o\text{-}phdn)(H_2O)_2]SO_4.10H_2O$, (D) $[Fe(CIP\text{-}o\text{-}phdn)(H_2O)_2](NO_3)_3.8H_2O$ and (E) $[Co(CIP\text{-}o\text{-}phdn)(H_2O)_2]Cl_2.7H_2O$.

A	B	C	D	E	Assignments
3533w	3522w 3478w	3341mbr	3545w	3375mbr	ν(O-H); H_2O; COOH
3156w 3044w	3133vw 3067vw 3040ms	3067vw 3009m	3044sh	3089vw 3022w	ν(C-H); aromatic
2943s 2867vw 2778m 2712ms	2955m 2843m 2767w 2722w 2712m	2889w 2766w 2711vw	2974m 2871vw 2800m 2758w	2955m 2822vw 2756w 2716m	ν(C-H); aliphatic
2650m 2611m 2465s	2644m 2615w 2465ms	2658vw 2554w	2689vw 2642vw 2507m	2650m 2611w 2465ms	ν(-NH_2^+)
1732vs	-	-	-	-	ν(C=O); COOH
-	1620vs	1620vs	1622m	1624s	$\nu_{as}(COO^-)$
1624vs	1589s	1574s	1574s	1570vs	ν(C=N)
1466vs	1539m 1494s 1474s	1485vs	1462vs	1485s	-CH; deformations of CH_2
1385m 1367sh	1377s	1393s 1378w	1385vs 1350sh	1393s	$\nu_s(COO^-)$ and $\nu(NO_3^-)$
1331ms	1322m	1304ms	1300m	1308s	$\delta_b(-CH_2)$
1261vs 1223w 1188m 1156m	1285s 1211sh 1172vw 1146ms	1269s 1189vw 1144w	1269s 1184m 1146m	1265s 1184m 1146m	ν(C-O), ν(C-N) and ν(C-C)
1089m 1034s	1034s	1103m 1072sh 1038m	1038ms	1107m 1034s	$\delta_r(-CH_2)$ $\nu(SO_4^{-2})$
989vw 968m 933m 887ms 833ms 822vw	989w 937ms 894w 867m 833ms	989vw 941ms 911vw 829m	945ms 899m 844m 822w 800vw	945s 895m 829m	-CH-bend; phenyl $\nu(NO_3^-)$
795m 767vw 748m 710m	783m 721s	779m 745s 710w	778w 752m 711m	778m 748s 710m	$\delta_b(COO^-)$
656w 622m 571ms 486m 478vw 436s	644vw 621ms 559s 494ms 436m	625ms 578m 544w 502m 478w 433w 413w	633ms 583m 544w 505s 422w	667vw 629m 578w 544m 502m 440w	ν(M-N), ν(M-O) and ring deformation

Keys: s = strong, w = weak, v = very, m = medium, br = broad, sh = shoulder, ν = stretching, δ_b = bending.

The infrared spectrum of CIP-o-phdn shows the absence of the bands attributable to ν(NH_2) group of o-phenylenediamine and ν(C=O) of ciprofloxacin. Instead, newly formed very strong band at 1624 cm^{-1} is obtained. This suggests the complete condensation of the amino groups with keto group [23] indicating the formation of the Schiff base linkage [24]. The IR spectra of all complexes containing hydration and or coordination water molecules display bands at 3545-3341 cm^{-1} due to ν(O−H) vibration mode of the water molecules [25] and this was confirmed by the results of thermal analysis. The stretching vibrations ν(C−H) of phenyl, −CH_2 and −CH_3 groups in these compounds are assigned as a number of bands in the region 3156–2711cm^{-1}. The assignments of all the C−H stretching vibrations agree quite well with the literature [26, 27].

The presence of a group of bands with different intensities in the range 2689–2465 cm^{-1}, which assigned to vibration of the quaternized nitrogen of the piperazine group, indicates the zwitterionic form of CIP-o-phdn is involved in the coordination to the investigated metal ions [27]. The two bands observed at 1732 and 1624 cm^{-1} in the spectrum of the CIP-o-phdn have been assigned to the stretching vibration of carboxylic ν(COOH) and the azomethine group ν(C=N), respectively [28-35]. The absent of the band at 1732 cm^{-1} in all complexes and the shift of the characteristic band of azomethine group to a lower value from 1624 cm^{-1} to 1589 cm^{-1} for Cr(III), at 1574 cm^{-1} for Mn(II) and Fe(III), at 1570 cm^{-1} for Co(II) indicated that the involvement of C=N group and one oxygen of the carboxylate group in the interaction with metal ion forming six and five membered rings and the carboxylic group is deprotonated [36].

In the case of monodentate carboxylate ligand, the antisymmetric and symmetric (COO^-) stretches will be shifted to higher and lower frequencies, respectively, with an average $\Delta\nu > 200$ cm^{-1} [37-43]. For our complexes the presence of band in the region 1624-1620 cm^{-1} in the IR spectra which assigned to the asymmetric stretching vibration ν_{as} of the ligated carboxylato group and the symmetric vibration occurs in the region 1393–1377 cm^{-1} with different intensities [24, 44, 45] and with $\Delta\nu > 200$ cm^{-1} indicated that the carboxylate group reacts as monodentate through one of oxygen atoms.

The spectra of the isolated solid complexes showed a group of bands with different intensities which characteristics for ν(M-N) and (M-O). The ν(M-N) and (M-O) bands observed at 559 and 494 cm^{-1} for Cr(III), at 625, 578, 544 and 502, cm^{-1} for Mn(II), at 633, 583, 544 and 505 cm^{-1} for Fe(III), at 629, 578, 544 and 502, cm^{-1} for Co(II) (Table 2) which are absent in the spectrum of CIP-o-phdn. This indicates the coordination of CIP-o-phdn through both C=N and carboxylic groups [25, 26].

Electronic spectra

The application of ultraviolet spectroscopy is more universal and can be useful in structural determinations of all chelates since they all absorb in this region [46]. The formation of the metal ciprofloxacin Schiff base complexes was also confirmed by the electronic solid reflection spectra. The electronic solid reflection spectra of CIP-o-phdn along with the Cr(III), Mn(II), Fe(III) and Co(II) complexes in the wavelength interval from 200 to 800 nm range are shown in Figure 1. It can be seen that free ciprofloxacin Schiff base reflected at 209, 229 and 321 nm (Table 3). The first two bands at 209 and 229 nm may be attributed to π-π^* transition and the second band observed at 321 nm is assigned to n-π^* transitions, these transitions occur in case of unsaturated hydrocarbons which contain ketone groups or azomethine group [32]. The shift of the reflection bands to higher values (bathochromic shift) and the absent of the band at 321 nm in case of Cr(III), Mn(II), Fe(III) and Co(II) complexes and presence of new bands in the reflection spectra of complexes indicate that the formation of their metal complexes [28]. The four complexes have bands in the range from 414 to 624 nm which may be assigned to the ligand to metal charge-transfer and d-d transition. [28-30, 47].

The spectrum of Schiff base Cr(III) complex show two absorption bands 624 and 569 nm which are assigned to $^6A_{2g} \longrightarrow {}^4T_{2g}(F)$ and $^4T_{2g} \longrightarrow {}^4T_{1g}(F)$ transitions, respectively, in favor of octahedral geometry [48]. The reflectance spectrum of Mn(II) complex showed two identified

bands at 619 and 571nm which are assigned to $^6A_{1g} \longrightarrow {}^4T_{1g}(^4G)$ and $^6A_{1g} \longrightarrow {}^4T_{2g}(^4G)$ transitions, respectively [49]. The electronic spectrum of octahedral Fe(III) complex show one absorption band at 533 nm which are assigned to $^6A_1 \longrightarrow {}^4T_2(G)$ transition [50]. The Schiff base Co(II) complex absorption spectrum show one absorption band at 623 nm which are assigned to $^4T_{1g}(F) \longrightarrow {}^4T_{1g}(P)$ transition in favor of octahedral geometry.

Finally, the results presented here, clearly indicated that the metal ions form stable solid complexes with CIP-o-phdn and monodentate ligand such as H_2O where metal ions are six coordinate.

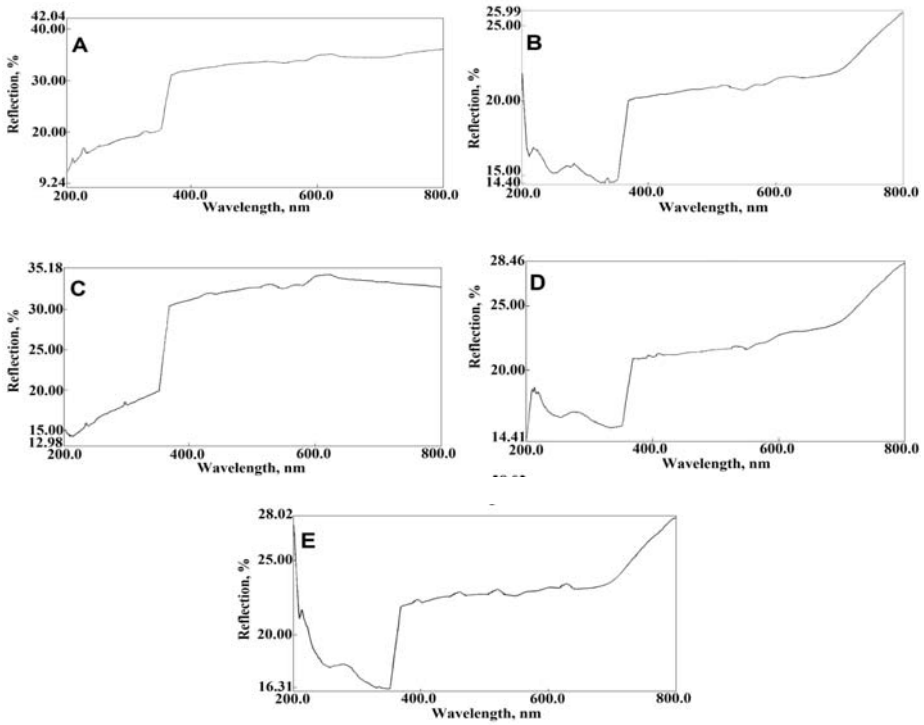


Figure 1. Electronic reflection spectra for (A) CIP-o-phdn; (B)$[Cr(CIP\text{-}o\text{-}phdn)(H_2O)_2](CH_3COO)_3.10H_2O$,(C)$[Mn(CIP\text{-}o\text{-}phdn)(H_2O)_2]SO_4.10H_2O$, (D) $[Fe(CIP\text{-}o\text{-}phdn)(H_2O)_2](NO_3)_3.8H_2O$ and (E) $[Co(CIP\text{-}o\text{-}phdn)(H_2O)_2]Cl_2.7H_2O$.

Table 3. UV-Vis. spectra of CIP-o-phdn; Cr(III), Mn(II), Fe(III) and Co(II).

Assignments (nm)	(CIP-o-phdn)	(CIP) Schiff base complex with			
		Cr(III)	Mn(II)	Fe(III)	Co(II)
π-π^* transitions	209, 229	219	234	213, 218	214
n-π^* transitions	321	274, 284, 337	293	277, 398	280, 398
Ligand-metal charge transfer	-	516	430, 520	414	456, 515
d-d transition	-	569, 624	571, 619	533	623

^{1}H NMR spectra

To make sure about the proposed structure of the isolated metal complexes ^{1}H NMR spectra were run. The ^{1}H NMR spectrum of CIP-o-phdn, in DMSO-d_6 (Table 4) showed the characteristic singlet at δ: 11 ppm to the proton of carboxylic (COOH). The resonance of the carboxylic proton (COOH) is not detected in the spectra of the isolated solid complexes that suggest the coordination of CIP-o-phdn through its carboxylato oxygen atoms [28-30, 51]. Also, the ^{1}H NMR spectra for complexes exhibit new peaks in the range 4.10-5.13 ppm, due to the presence of water molecules in the complexes. On comparing main peaks of CIP-o-phdn with its complexes, it is observed that all the peaks of the free ligand are present in the spectra of the complexes with chemical shift upon binding of CIP-o-phdn to the metal ion [52].

Table 4. ^{1}H NMR values (ppm) and tentative assignments for (A) CIP-o-phdn; (B) [Cr(CIP-o-phdn)$(H_2O)_2$]$(CH_3COO)_3.10H_2O$, (C) [Mn(CIP-o-phdn)$(H_2O)_2$]$SO_4.10H_2O$, (D) [Fe(CIP-o-phdn)$(H_2O)_2$]$(NO_3)_3.8H_2O$ and (E) [Co(CIP-o-phdn)$(H_2O)_2$]$Cl_2.7H_2O$.

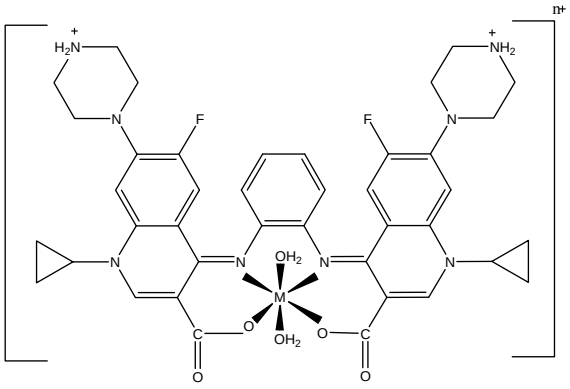
A	B	C	D	E	Assignments
1.21-1.34	1.19-1.30	0.82-1.62	1.23	1.05-1.29	δH,-CH_2 and -CH cyclopropane
2.09	2.09	2.09	2.09	2.08-2.26	δH, -NH; piperazine
2.49	2.50	2.51	2.50	2.49- 2.73	δH, -$^+NH_2$
3.32-3.86 -	3.01-3.83 4.40	3.44 5.13	3.33 4.10	3.33-3.77 4.10	δH, -CH_2 aliphatic δH H_2O
7.60- 9.11	7.57- 8.67	7.69-8.69	7.88-8.30	7.85-8.65	δH, -CH aromatic
11	-	-	-	-	δH, -COOH

Thermal studies

Thermogravimetric (TGA) and differential thermogravimetric (DTG) analyses for CIP-o-phdn and their isolated solid complexes were carried out to get information about the thermal stability of these new complexes and to suggest a general Scheme for thermal decomposition as well as to ascertain the nature of associated water molecules. In the present investigation, heating rates were suitably controlled at 10 ^oC min^{-1} under nitrogen atmosphere and the weight loss is measured from the room temperature to 800 ^oC. Figure 2 represent the TGA and DTG curves and Table 5 gives the maximum temperature values for decomposition along with the corresponding weight loss values for each step of the decomposition reaction. These data support the proposed complexes chemical formulae. CIP-o-phdn is thermally stable at room temperature and the decomposition started at 35 ^oC and finished at 715 ^oC with one stage at three maxima 200, 311 and 630 ^oC and is accompanied by a weight loss of 99.82%.

The thermal decompostion of [Cr(CIP-o-phdn)$(H_2O)_2$]$(CH_3COO)_3.10H_2O$ complex proceeds with two main degradation steps. The first stage of decomposition occurs at a temperature maximum of 60 ^oC. The found weight loss associated with step is 15.19% and may be attributed to the loss of the ten water molecules which is in good agreement with the calculated values of 15.27%. Loss of water crystallization at a relatively low temperature may indicate weak H-bonding involving the H_2O molecule and the complex. The second stage of decomposition occurs at three maxima 323, 400 and 650 ^oC with intermediate formation of very unstable products which were not identified [25, 53, 54] and the weight loss found at this stage equals 76.34% corresponds to loss $20C_2H_2+3.5N_2+NO+2HF+4CO+5.5H_2O$. For Mn(II) complex the thermal decomposition exhibits two main degradation steps. The first step of decomposition occurs from 25 to 282 ^oC is accompanied by a weight loss of 16.28% in agreement with the theoretical values 16.35% for the loss of ten uncoordinated water molecules (water of

crystalization). The second step of decompostion occurs at four maxima 282, 332, 400 and 600 ^{o}C with a weight loss of 68.68% this associated with the loss of ciprofloxacin Schiff base forming manganize sulphate as a final product (Table 5). The thermal degradation for the $[Fe(CIP\text{-}o\text{-}phdn)(H_2O)_2](NO_3)_3.8H_2O$ exhibits two degradation steps. The first step of decomposition occurs in the range 25-175 ^{o}C, with one maximum temperature at 75 ^{o}C, is accompanied by a weight loss of 12.34% corresponding exactly to the loss of eight water molecules. The second step of degradation occurs at three maxima at 231, 356 and 438 ^{o}C and is accompanied by a weight loss of 80.46%, corresponding to the loss of $19C_2H_2$+10NO+2HF+0.5N_2+0.5H_2+2CO+1.5H_2O. The actual weight loss from these two steps is 92.80%, close to the calculated value 93.09%. The $[Co(CIP\text{-}o\text{-}phdn)(H_2O)_2]Cl_2.7H_2O$ complex decomposes in two steps within the temperature range 25-900 ^{o}C with total mass loss 93.00% leaving CoO as residue. The proposed structure formula on the basis of the results discussed in our paper located as follows (Scheme 1).



M = Cr(III), Mn(II), Fe(III) and Co(II)
N = 2 for Mn(II), Co(II) and 3 for Cr(III), Fe(III)

Scheme 1. The coordination mode of M with CIP-o-phdn.

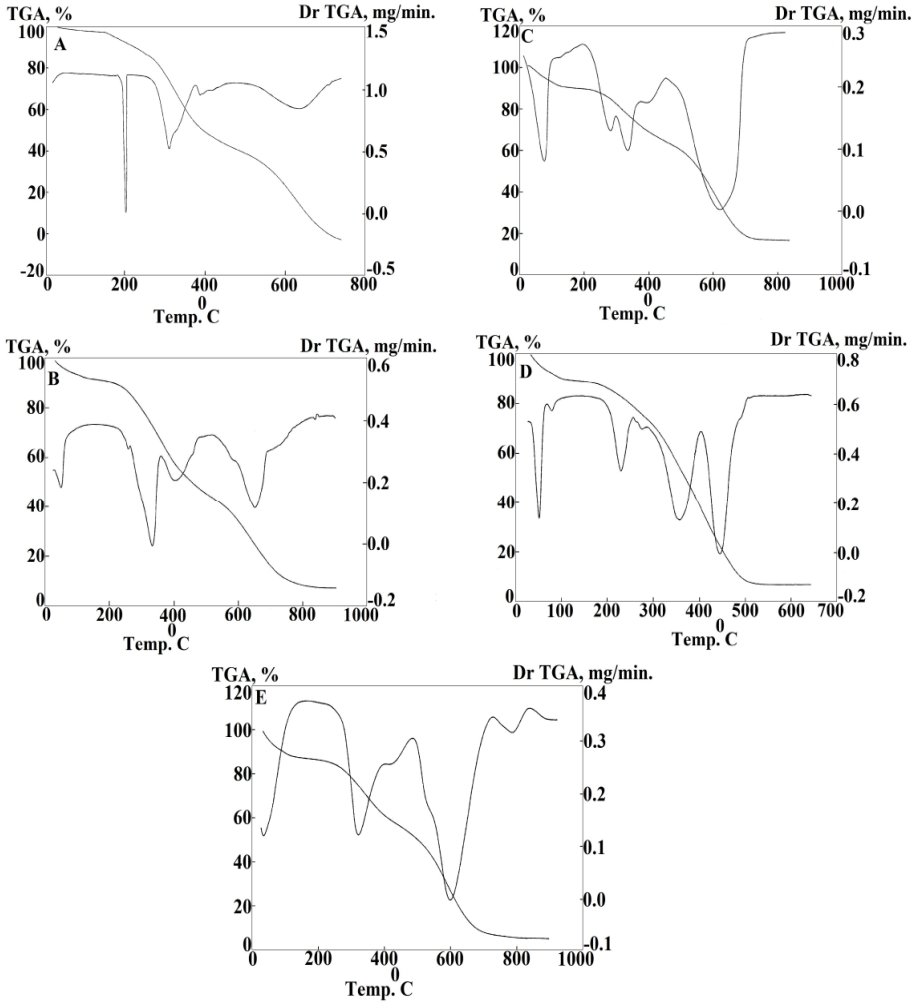


Figure 2. TGA and DTG diagrams for (A) CIP-o-phdn; (B)[Cr(CIP-o-phdn)$(H_2O)_2$] $(CH_3COO)_3.10H_2O$,(C)[Mn(CIP-o-phdn)$(H_2O)_2$]$SO_4.10H_2O$, (D) [Fe(CIP-o-phdn) $(H_2O)_2$]$(NO_3)_3.8H_2O$ and (E) [Co(CIP-o-phdn)$(H_2O)_2$]$Cl_2.7H_2O$.

Mass spectra

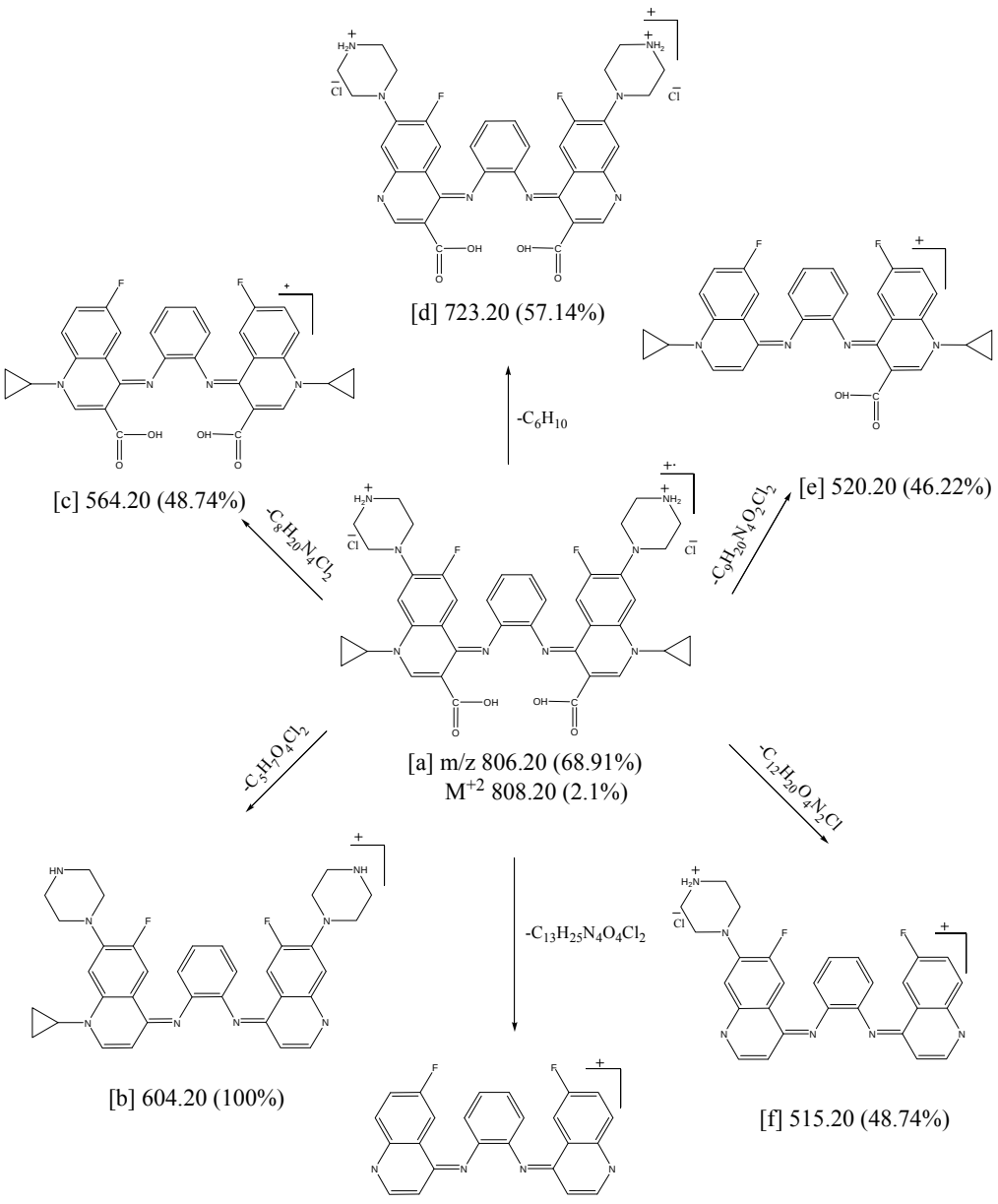
The idea of mass spectrometer builds up on the separation of fragments ions dependent to the variation of these ions with the ratio of mass to charge (m/z) [55]. Mass spectrum of the synthesized CIP-o-phdn was in a good agreement with the suggested structure (Scheme 2). CIP-o-phdn showed molecular ion peak (M^{+}) at m/z = 806.2 (68.91%), and M^{+2} at m/z = 808 (2.1%). The molecular ion peak [a] gave fragment which refer to base peak [b] at m/z = 604.20 (100%). The molecular ion peak [a] losses $C_8H_{20}N_4Cl_2$ to give fragment [c] at m/z = 564.20 (48.74%) and it losses C_6H_{10} to give fragment [d] at m/z = 723.20 (57.14%). It loses $C_9H_{20}N_4O_2Cl_2$ to give [e] at m/z = 520.20 (46.22%). The molecular ion peak [a] gave fragment [f] at m/z = 515.20

(48.74%) and also [g] at m/z = 435.20 (70.59%). The mass spectrum of Co(II) complex displayed molecular peak at 1024.9 which refer to M.Wt. of the complex with the abundance 10.10%. For the other three complexes Cr(III), Mn(II) and Fe(III) with the calculated molecular weights 1179, 1101 and 1155.8, respectively, according to the qualitative and thermogravimetric analyses, the molecular peaks are found outside the scale of the instrument.

Table 5. The maximum temperature T_{max} (^oC) and weight loss values of the decomposition stages for CIP-o-phdn, Cr(III), Mn(II), Fe(III) and Co(II).

Compounds	Decompo-sition	T_{max} (^oC)	Weight loss (%)		Lost species
			Calc.	Found	
(CIP-o-phdn) $C_{40}H_{42}N_8O_4F_2Cl_2$)	First step Total loss Residue	200, 311, 630	100 100	99.82 99.82	$18C_2H_2$+4CO+2HCl+2HF+H_2+$4N_2$
[Cr(CIP-o-phdn)$(H_2O)_2$]$(CH_3COO)_3$ $10H_2O$ $C_{46}H_{73}N_8O_{22}F_2Cr$)	First step Second step Total loss Residue	60 323, 400, 650	15.27 76.25 91.52 8.48	15.19 76.34 91.53 8.47	$10H_2O$ $20C_2H_2$+3.5N_2+NO+2HF+4CO+ 5.5H_2O $CrO_{1.5}$+2C
[Mn(CIP-o-phdn)$(H_2O)_2$]SO_4. $10H_2O$ $C_{40}H_{64}N_8O_{20}F_2SMn$)	First step Second step Total loss Residue	64, 282, 332, 400, 600	16.35 68.85 85.20 14.8	16.28 68.68 84.96 15.04	$10H_2O$ $19C_2H_2$+3NO+CO+2HF+$2H_2O$+2.$5N_2$ $MnSO_4$+C
[Fe(CIP-o-phdn)$(H_2O)_2$]$(NO_3)_3$. $8H_2O$ $C_{40}H_{60}N_{11}O_{23}F_2Fe$)	First step Second step Total loss Residue	75 231, 356, 438	12,45 80.64 93.09 6.91	12.34 80.46 92.80 7.20	$8H_2O$ $19C_2H_2$+10NO+2HF+0.5N_2+0.5H_2+2CO+ 1.5H_2O $FeO_{1.5}$
[Co(CIP-o-phdn)$(H_2O)_2$]Cl_2.$7H_2O$ ($C_{40}H_{58}N_8O_{13}F_2Cl_2Co$)	First step Second step Total loss Residue	55 314, 586, 759	12.28 80.42 92.70 7.30	12.30 80.70 93.00 7.00	$7H_2O$ $20C_2H_2$+5NO+2HF+2HCl+1.$5N_2$ CoO

.



Scheme 2. Fragmentation pattern of CIP-o-phdn.

Biological activity

The susceptibility of certain strains of bacterium, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal screening was studied against two species *Candida albicans* and *Aspergillus fumigates* towards CIP-o-phdn, metal salts and its complexes was judged by measuring size of the inhibition diameter (Table 6 and Figure 3). As assessed by color, the complexes remain intact during biological testing. A comparative study of ligand and their metal complexes showed that the metal complexes exhibit higher antibacterial activity for Gram-positive and Gram-negative. Fe(III) is very highly significant for *Escherichia coli* and *Pseudomonas aeruginosa* and highly significant for *Bacillus subtilis* but Mn(II) showed moderated antibacterial activity for *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and highly significant for *Staphylococcus aureus*, and no antifungal activity observed for ligand and their metal complexes (Table 6). The results are promising compared with the previous studies [51, 53, 56, 57]. Such increased activity of metal chelate can be explained on the basis of the oxidation state of the metal ion, overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antimicrobial activity. On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes [51]. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the microorganisms. Finaly, the data indicated that CIP-o-phdn exhibits higher antibacterial activity compared with free ciprofloxacin [53].

Table 6. The inhibition diameter zone values (mm) for (CIP-o-phdn) and its metal complexes.

Tested compounds		Microbial species					
		Bacteria				Fungi	
		E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*C. albicans*	*A. fumigatus*
CIP		27±0.35	23±0.11	32±0.22	26±0.40	0	0
CIP-o-phdn		30±0.33	25±0.11	36±0.22	29±0.90	0	0
$[Cr(CIP\text{-}o\text{-}phdn)(H_2O)_2](CH_3COO)_3.10H_2O$		40^{+1}±0.22	44^{+3}±0.02	46^{+1}±0.44	48^{+2}±0.06	0	0
$[Mn(CIP\text{-}o\text{-}phdn)(H_2O)_2]SO_4.10H_2O$		41^{+1}±0.11	35^{+1}±0.03	45^{+1}±0.30	50^{+2}±0.88	0	0
$[Fe(CIP\text{-}o\text{-}phdn)(H_2O)_2](NO_3)_3.8H_2O$		50^{+3}±0.22	45^{+3}±0.04	52^{+2}±0.20	44^{+1}±0.33	0	0
$[Co(CIP\text{-}o\text{-}phdn)(H_2O)_2]Cl_2.7H_2O$		39^{+1}±0.15	43^{+3}±0.05	50^{+2}±0.13	50^{+2}±0.11	0	0
$Cr(OCOCH_3)_3$		0	0	0	0	0	0
$MnSO_4.6H_2O$		0	0	0	0	0	0
$Fe(NO_3)_3.9H_2O$		10±0.33	12±0.11	0	0	0	0
$CoCl_2.6H_2O$		0	0	0	0	0	0
Control (DMF)		0	0	0	0	0	0
o-Phenylenediamine		15±0.33	0	0	0	0	0
Standard	Ampicilin	0	0	28±0.40	0	0	0
	Amoxycilin	0	0	22±0.11	18±1.73	0	0
	Cefaloxin	24±0.34	0	27±1.15	16±0.52	0	0

Statistical significance P^{NS} P not significant, $P > 0.05$; P^{+1} P significant, $P < 0.05$; P^{+2} P highly significant, $P < 0.01$; P^{+3} P very highly significant, $P < 0.001$; student's *t*-test (paired).

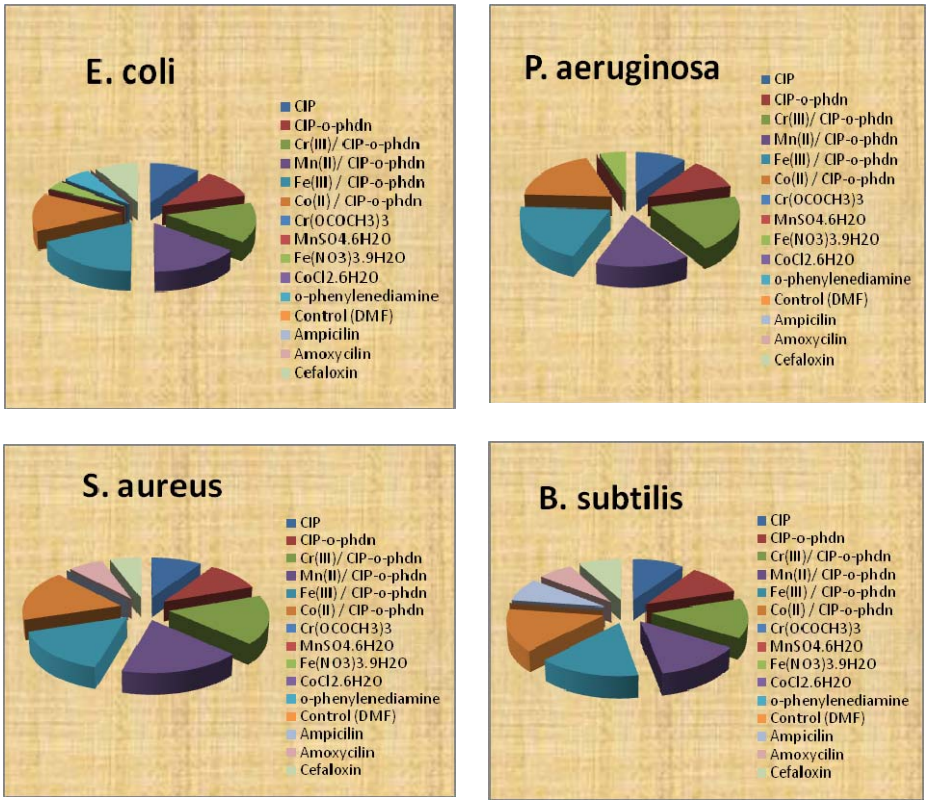


Figure 3.Statistical representation for biological activity of CIP-o-phdn and its metal complexes.

CONCLUSION

To summarize the new CIP-o-phdn tetradentate ligand (N,N'-o-phenylene bis 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid) has been synthesized and their coordination chemistry with Cr(III), Mn(II), Fe(III) and Co(II) has been studied. The four new complexes were characterized with physicochemical and spectroscopic methods. In all these complexes, CIP-o-phdn is on deprotonated mode and acts as tetradentate ligand bound to the metal ion through two oxygen atoms of carboxylate groups and two nitrogen atoms of azomethine groups. Each metal is six coordinate and the geometry described as distorted octahedral. The antibacterial activity of CIP-o-phdn is higher than ciprofloxacin alone.

REFERENCES

1. Dhar, D.N.; Taploo, C.L. *J. Sci. Ind. Res.* **1982**, 41, 501.
2. Przybylski, P.; Huczynski, A.; Pyta, K.; Brzezinski, B.; Bartlf. *Curr. Org. Chem.* **2009**, 13, 124.
3. Shaw, A.Y.; Chang, C.Y.; Hsu, M.Y.; Lu, P.J.; Yang, C.N.; Chen, H.L. *Eur. J. Med. Chem.* **2010**, 45, 2860.

4. Bringmann, G.; Dreyer, M.; Faber, J.H.; Dalsgaard P.W.; Staerk, D.; Jaroszewski, J.W. *J. Nat. Prod.* **2004**, 67, 743.
5. De Souza, A.O.; Galetti, F.C.S.; Silva, C.L.; Bicalho, B.; Parma, M.M.; Fonseca, S.F. *Quim. Nova*. **2007**, 30, 1563.
6. Guo, Z.; Xing, R.; Liu, S.; Zhong, Z.; Ji, X.; Wang, L. *Carbohydr. Res*. **2007**, 10, 1329.
7. Khalil, S.M.E. *Chem. Pap*. **2000**, 54, 12.
8. Hamilton, D.E.; Drago, R.S.; Zombeck, A. *J. Am. Chem. Soc.* **1987**, 109, 374.
9. Chen, D.; Martel, A.E. *J. Inorg. Chem.* **1987**, 26, 1026.
10. Nishinaga, A.; Yamada, T.; Fujisawa, H.; Ishizaki, K. *J. Mol. Catal.* **1988**, 48, 249.
11. Xi, Z.; Liu, W.; Cao, G.; Du, W.; Huang, J.; Cai, K.; Guo, H. *Cuihua. Xuobao*. **1986**, 7, 357.
12. Chakraborty, H.; Paul, N.; Rahman, M.L. *Trans. Met. Chem.* **1994**, 19, 524.
13. Zhao, Y.D.; Pang, D.W.; Zong, Z.; Cheng, J.K.; Luo, Z.F.; Feng, C.J.; Shen, H.Y.; Zhung, X.C. *Huaxue. Xuebao*. **1988**, 56, 178.
14. Sreekala, R.; Yusuff, K.K.; Mohammed, K.K. *Catal. Pap.* **1994**, 507.
15. Ramesh, R.; Sugantyhy, P.K.; Natarajan, K. *Synth. React. Inorg. Met.–Org. Chem.* **1996**, 26, 47.
16. Ohashi, Y. *Bull. Chem. Soc. Jpn.* **1997**, 70, 1319.
17. Teleb, S.M.; Sadeek, S.A.; Nour, E.M. *Spectrosc. Lett.* **1993**, 26, 169.
18. Nour, E.M.; Al-kority, A.M.; Sadeek, S.A.; Teleb, S.M. *Synth. React. Inorg. Met.-Org. Chem.* **1993**, 23, 39.
19. Vogel, A.I. *"Qualitative Inorganic Analysis",* 6th ed., Wiley: New York; **1987**.
20. Beecher, D.J.; Wong, A.C. *Appl. Environ. Microbial.* **1994**, 60, 1646.
21. Fallik, E.; Klein, J.D.; Grinberg, S.; Lomaniec, E.; Lurie, S.; Lalazar, A. *J. Econ. Entomol.* **1993**, 77, 985.
22. Geary, W.J. *Coord. Chem. Rev.* **1971**, 7, 81.
23. Chandra, S.; Kumar U. *J. Saudi. Chem. Soc.* **2004**, 8, 77.
24. Keypour, H.; Shayesteh, M.; Sharifi-Rad, A.; Saleh Zadeh, S.; Khavasi, H.; Valencia, L. *Organomet. Chem.* **2008**, 693, 3179.
25. Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 4th ed., Wiley: New York; **1986**; p 230.
26. Sadeek, S.A.; Teleb, S.M.; Refat, M.S.; Elmosallamy, M.A.F. *J. Coord. Chem*. **2005**, 58, 1077.
27. Silverstein, R.M.; Bassler, G.C.; Morril, T.C. *Spectroscopic Identification of Organic Compounds*, 5th ed., Wiley: New York; **1991**.
28. Turel, I.; Bukovec, P.; Quirós, M. *Int. J. Pharm.* **1997**, 152, 59.
29. Sadeek, S.A.; Refat, M.S.; Hashem, H.A. *J. Coord. Chem.* **2006**, 59, 759.
30. Sadeek, S.A.; EL-Shwiniy, W.H. *J. Mol. Struct.* **2010**, 977, 243.
31. Sadeek, S.A.; EL-Shwiniy, W.H. *J. Mol. Struct.* **2010**, 981, 130.
32. Gao, F.; Yang; P.; Xie; J.; Wang, H. *J. Inorg. Biochem*. **1995**, 60, 61.
33. Sadeek, S.A.; EL-Shwiniy, W.H.; Zordok, W.A.; EL-Didamony, A.M. *Spectrochim. Acta Part A* **2011**, 78, 854.
34. Sadeek, S.A.; El-Attar, M.S.; Abd El-Hamid, S.M. *J. Mol. Struct*. **2013**, 1051, 30.
35. Sadeek, S.A.; El-Attar, M.S.; Abd El-Hamid, S.M. *Synth. React. Inorg. Met.-Org. Nano-Metal Chem*. **2015**, 45, 1412.
36. Refat, M.S. *Spectrochim. Acta* **2007**, 68, 1393.
37. Kessissoglou, D.P.; Manoussakis, G.E.; Hatzidimitriou, A.G.; Kanatzidis, M.G. *Inorg. Chem*. **1987**, 26, 1395.
38. Davies, J.A.; Eagle, C.T.; Pinkerton, A.A.; Syed, R. *Acta Cryst.* **1987**, 43, 1547.
39. Gregg, M.R.; Powell, J.; Sawyer, J.F. *Acta Cryst*. **1988**, 44, 43.
40. Robertson, G.B; Tucker, P.A. *Acta Cryst*. **1983**, 39, 858.

41. Bryndza, H.E.; Calabrese, J.C.; Marsi, M.; Roe, D.C.; Tam, W.; Bercaw, J.E. *J. Am. Chem. Soc*. **1986**, 108, 4805.
42. Manoussakis, G.; Bolos, C.; Ekateriniadou, L.; Sarris, C. *Eur. J. Med. Chem*. **1987**, 22, 421.
43. Kortsaris, A.E.; Kyriakidis, D.A. *Microbiologica* **1988**, 11, 347.
44. Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, Wiley: New York; **1963**; p 232.
45. Deacon, G.B.; Phillips, R. *Coord. Chem. Rev.* **1980**, 33, 227.
46. Nakamoto, K.; McCarthy, P.J.; FuJiwara, S.; Shimura, Y.; Fujita, J.; Hare, C.R.; Saito, Y. *Spectroscopy and Structure of Metal Chelate Compounds*, John Wiley and Sons, Inc.: New York; **1968**.
47. Cotton, F.A.; Wilkinson, G.; Murillo, C.A.; Bochmann, M. *Advanced Inorganic Chemistry*, 6th ed., Wiley: New York; **1999**.
48. Dubey, R.K.; Dubey, U.K.; Mishra, C.M. *Indian J. Chem. A* **2008**, 47, 1208.
49. Lever, A.B.P. *Coord. Chem. Rev*. **1968**, 3, 119.
50. Mondal, N.; Dey, D.K.; Mitra, S.; Abdul Malik, K.M. *Polyhedron* **2000**, 19, 2707.
51. Muhammad, I.; Javed, I.; Shahid, I.; Nazia, I. *Turk. J. Biol.* **2007**, 31, 67.
52. Skauge, T.; Turel, I.; Sletten, E. *Inorg. Chem. Acta* **2002**, 339, 239.
53. Sadeek, S.A.; EL-Shwiniy, W.H.; Zordok, W.A.; EL-Didamony, A.M. *Spectrochim. Acta (A)* **2011**, 78, 854.
54. Brzyska, W.; Hakim, M. *Polish J. Chem.* **1992**, 66, 413.
55. Sanmartin, J.; Novio, F.; Garcia-Deibe, A.M.; Fondo, M.; Ocampo, N.; Bermejo, M.R. *Polyhedron* **2006**, 25, 1714.
56. Hughes, M.N. *The Inorganic Chemistry of Biological Processes*, 2nd ed., Wiley Interscience: New York; **1981**.
57. Rossmore, H.W.; Block, S.S. (Eds.) *Disinfection, Sterilization and Preservation*, 4th ed., Lea and Febinger: Philadelphia; **1991**; p 290.