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Mouhaned Y. Al-Darwesh, and Kaiss R. Ibraheem



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Synthesis and Characterization of Some Metal Ions Derived from Ciprofloxacin and Evaluation of their Biological Activity

Mouhaned Y. Al-darwesh^{a)} and Kaiss R. Ibraheem^{b)}

Department of Chemistry, College of Science University of Anbar, Anbar, Iraq

^aCorresponding author: myturky@uoanbar.edu.iq

^bSc.kaiss1966@uoanbar.edu.iq

Abstract. This study aims to prepare and characterize a new ligand (LT). This ligand which is derived from ciprofloxacin is able for coordination with the following metal ions: Cu (II), Co (II), Fe (III), Zn (II), Cr (III), Ni (II), and Mn (II); where: LT = 4- (3-carboxy-4 - ((3-carboxy-1-cyclopropyl-6-fluoro-7 (piperazine-1yl) quinoline-4 (1h) -ylidene) -6-fluoro-4-1-hydroquinone -7-yl) piperazine-1- were investigated. Analysis of elements, electrical conductivity, magnetic characteristics, melting point, UV-Vis, infrared (IR), ¹H nuclear magnetic resonance spectrum, mass spectroscopy, and thermal analysis were employed to describe the synthesized complexes. The TGA has been checked to know the chemical composition of compounds. The measured molar conductivity values referred to that the non-electrolytic complexes were evaluated to investigate the biological efficacy of the ligand and the synthesized complexes toward the obtained bacteria.

INTRODUCTION

The Ciprofloxacin is an antibiotic belonging to the quinolones family. It is considered to be highly successful, especially in the cases of contamination by sensitive bacteria in the respiratory and urinary tracts [1]. Quinolone, on the other hand, is one of antibiotics groups that have been widely employed in the treatment of many bacterial infections [2]. Drug complexes are highly biologically active and widely used today because of their significant role in the synergistic process of certain drugs and their useful biological activity. [3] The association of metallic and non-metallic ions has been found to increase the viability of drugs and enhance their efficacy [4], making them more active than in the same ligand [5]. The complexes of ciprofloxacin are highly effective against bacteria, fungi, viruses and cancer cells [6]. Therefore, antibacterial agents are receiving a great deal of attention at the present time and are witnessing a great and rapid development due to the resistance of bacteria to the old traditional medicines, where they become more resistant to them [7]. Antibiotics ciprofloxacin and Norfloxacin are among the broad range of Quinolones which are effective toward gram positive and gram negative bacteria [8]. The resistance of antagonists has evolved by modifying the antigen target. One of the most important factors that have led to the emergence of multiple antibiotic resistances is the DNA genes, which transform supercoiled bacterium [9] due to pump influx. Thus, the flow of antibiotics reduces the concentration of antibiotics at the target sites on which the antibiotic works [10].

MATERIALS AND METHODS

The used chemicals in this research were purchased from the commercial markets, including CrCl₂.6H₂O, COCl₂, 6H₂O, MnCl₂. 4H₂O, FeCl₃.6H₂O CuCl₂.2H₂O ZnCl₂, and Ethanol absolute from BDH. Other chemicals including NaOH, Ciprofloxacin hydrochloride, DMF and DMSO were obtained from Sigma Aldrich. Elemental analyses of the following elements C, N, and H have been achieved by using a C.H.N Euro Vector (model EA3000, (single) V. 3.0 elemental analyzer). The metal ions contents were calculated as oxides of metals. Measurements of molar

conductivity of the ligand and its complex in the solvent DMSO at 10^{-3} M have been achieved by Seven HANNAEC214. The magnetic measurements have been achieved on the Sherwood Scientific magnetic balance by using method of Gouy. The spectrum of electron impact (70 EV) mass was reported using the model of Finnegan MAT (Mass spectra LC-MSQP50A: Shimadzu (E170Ev) spectrophotometer. The UV-visible spectrum was obtained in the solution of DMSO (10^{-3} M) for the ligand and the prepared complex from it by the spectrophotometer (Shimadzu -160A) using quartz cells (1 cm), at wavelengths from 200 to 1000 nm. Infrared (IR) spectrum was measured by FTIR spectrophotometer (Shimadzu 8400 S) at wavelength region $400-4000\text{ cm}^{-1}$ as disks of KBr. The spectrum of $^1\text{H NMR}$ was measured by a varian400 MHz in the solvent of DMSO d_6 ; while the chemical shift was measured near peaks of solvent. The thermal analysis (TG) has been achieved in a dynamic nitrogen atmosphere (20 ml per min) at a rate of heating 10°C per minute by the thermal analyzer (Perkin Elmer TG 4000).

Synthesis of the ligand(LT) =4-(3-carboxy-4-(((3-carboxy-1-cyclopropyl-6-fluoro-7-(piperazin-1-yl) quinolin-4(1H)-ylidene)carbamothioyl)imino)-1-cyclopropyl-6-fluoro-1,4-dihydroquinolin-7-yl)piperazin-1-ium

In the round-bottom flask (100 ml) put 7.71 g (0.02mol) of ciprofloxacin dissolved in 30ml of absolute ethanol. Stir the solution using a magnetic stirrer until dissolution completed. Then, add 0.76 g (0.01mol) of thiourea dissolved in 10 ml of the absolute ethanol. At constant stirring, drop it into the solution then add 5 drops of glacial acetic acid to the mixture and heat up (Reflux) for 4 h, let the mixture to cool and form a white tanned precipitate. Then, nominate the precipitate, dry and recrystallize it in absolute ethanol to obtain ligand in its pure form. The preparation ratio was 2: 1.

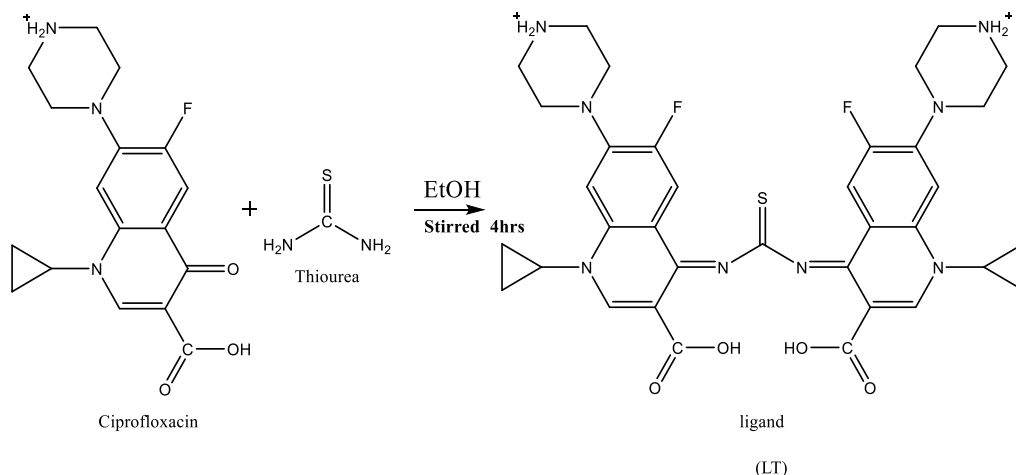
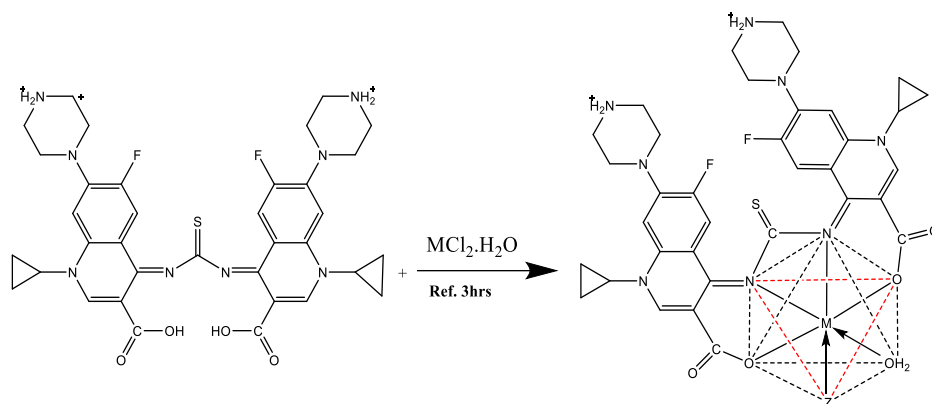


FIGURE 1. Synthesis approach of the ligand (LT)

Synthesis of metallic complexes

In a one hundred millilitres round-bottom flask, put 0.702 g (0.001mol) of LT dissolved in 20 ml of absolute ethanol and add 0.04 g of NaOH. Preheat the mixture in a water bath until dissolution. Add the mixture to a solution of dissolving (0.001mol) 0.230 g of the metal salt $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ in a small volume of absolute ethanol 10 ml. Preheat the final mixture in a water bath until dissolve the salt, mix the two solutions and heated (Reflux) in a water bath for three hours. Isolate the precipitated colour (brown) and recrystallize it from absolute ethanol. In the same way, complexes was prepared and the ratio of preparation was (M: L) (1:1).



Ligand M= Cu (II), Co (II), Fe (III), Zn (II), Cr (III), Ni (II), and Mn (II) complex
(LT) Z= Cl or H₂O

FIGURE 2. Scheme of production of the metallic complex

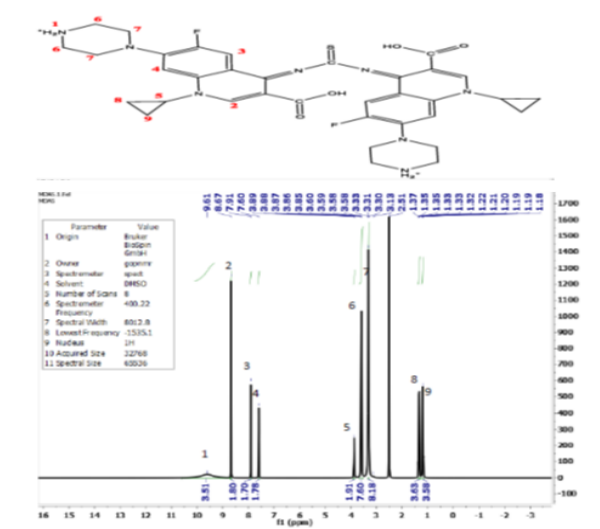


FIGURE 3. ¹H-NMR spectra of LT

TABLE1. Analytical data and physical characteristics of ligand and its metallic complexes

The Compounds	Molecular Wt.	Yield (%)	Melting P. (°C)	Colour	Found (calcd.) (%)				
					C	H	N	M	Cl
C ₃₅ H ₃₆ F ₂ N ₈ O ₄ S (LT)	702.78	83	308	White	(59.65) (59.47)	(5.43) (5.17)	(15.90) (15.66)	-	-
C ₃₅ H ₃₆ ClCrF ₂ N ₈ O ₅ S [Cr(LT)(H ₂ O)Cl]	806.23	71	299	Dark Brown	(52.14) (51.96)	(4.50) (4.11)	(13.90) (13.45)	(6.45) (6.11)	(4.40) (4.19)
C ₃₅ H ₃₆ ClCoF ₂ N ₈ O ₅ S [Co(LT)(H ₂ O) ₂]	813.16	70	319	Dark Blue	(51.70) (51.23)	(4.46) (4.10)	(13.78) (13.26)	(7.25) (7.02)	(4.36) (4.14)
C ₃₅ H ₃₆ F ₂ MnN ₈ O ₅ S [Mn(LT)(H ₂ O) ₂]	773.19	68	318	Light Yellow	(51.33) (51.14)	(4.69) (4.23)	(14.48) (13.94)	(7.10) (6.86)	-
C ₃₅ H ₃₆ ClF ₂ FeN ₈ O ₅ S [Fe(LT)(H ₂ O)Cl]	810.08	77	331	Dark Orange	(51.89) (51.26)	(4.48) (4.03)	(13.83) (13.19)	(6.89) (6.31)	-
C ₃₅ H ₃₆ CuF ₂ N ₈ O ₅ S [Cu(LT)(H ₂ O) ₂]	782.33	69	303	ellowish green	(53.74) (53.14)	(4.64) (4.23)	(14.32) (13.89)	(8.12) (7.79)	-
C ₃₅ H ₃₆ F ₂ N ₈ O ₅ SZn [Zn(LT)(H ₂ O) ₂]	784.16	66	325	White	(53.61) (53.20)	(4.63) (4.14)	(14.29) (13.76)	(4.85) (4.27)	-
C ₃₅ H ₃₆ F ₂ N ₈ NiO ₅ S [Ni(LT)(H ₂ O) ₂]	777.19	68	314	Green	(54.07) (53.69)	(4.67) (4.13)	(14.41) (13.75)	(7.55) (6.84)	-

RESULTS AND DISCUSSION

Tetradentate complexes have been obtained from the reaction between Schiff base and metal ions at the molar ratio (1:1). The prepared Schiff base ligand and their complexes were very stable in the solid-state at the room temperature. Most complexes and ligand are dissolved in solvents of DMSO and DMF.

The Spectrum of $^1\text{H-NMR}$ of the Ligand (LT)

NMR spectrum was used to diagnose LT using DMSO- d_6 as a solvent. The LT spectrum shown in Figure 3 showed two signals at δ (9.61) ppm and δ (8.67) ppm belonging to the carboxylic group COOH protons and while the multiplet signal within the range of ppm δ (7.60-7.91) belonging to the protons of aromatic rings, while the protons of the aliphatic ring have multiple signals at δ (3.58, 3.31) ppm. Signals at displacements δ (3.85, 1.35, 1.32) ppm are also due to cyclopropan ring protons. Table 2 shows the ligand $^1\text{H-NMR}$ spectrum data (LT).

TABLE 2. NMR spectrum ($^1\text{H-NMR}$) in solvent (DMSO- d_6) ligand (LT)

Ligand(LT) peaks	Assignments
9.61	δH , COOH
8.67	δH , NH, piperazine
(7.91-7.60)	δH , -CH aromatic
(3.58, 3.31)	δH , -CH aliphatic
(3.85, 1.35, 1.32)	δH , -CH cyclopropane

The Mass Spectrum

The mass spectrum of some complexes (peaks at 796 m/z, and 803 m/z) represent the molecular weight of the complexes respectively. It is additional proof of the correctness of the proposed complexes as shown in Figures (4-5).

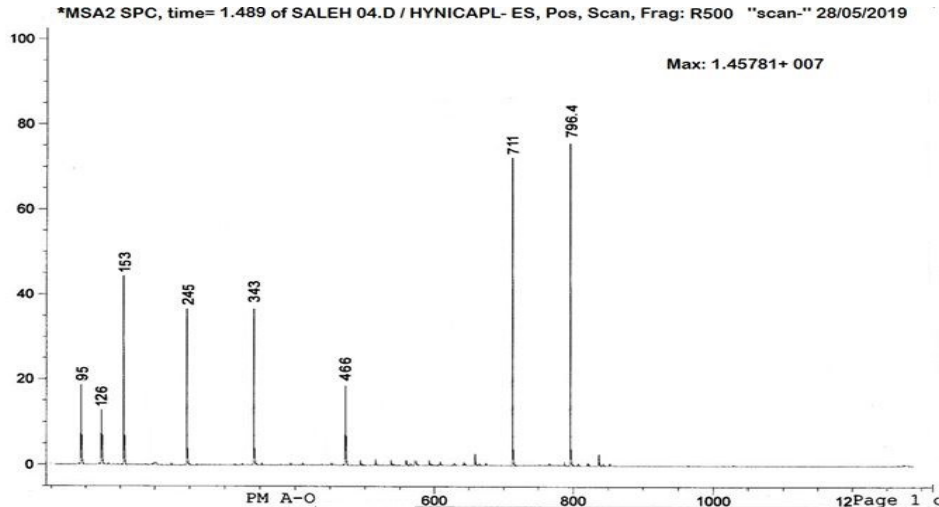


FIGURE 4. Mass Spectrum of $[\text{Cr}(\text{LT})(\text{H}_2\text{O})\text{Cl}]$.

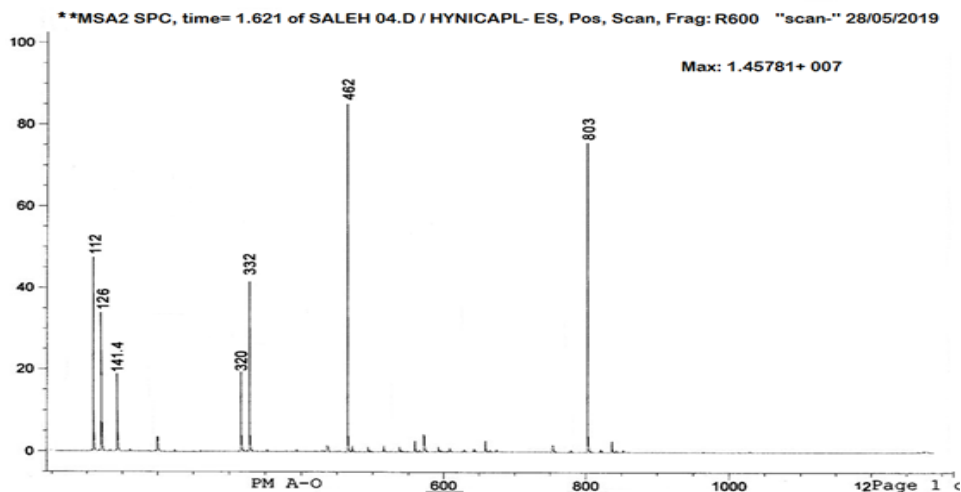


FIGURE 5. Mass Spectrum of $[\text{Co}(\text{LT})(\text{H}_2\text{O})_2]$

Studies of Infrared Spectra

Electronic techniques in the analysis and study of ligand and their complexes prepared within the range between 4000-400 cm^{-1} and then in the vibrational spectra [11]. In this research, infrared technology has been used to diagnose the prepared complexes in comparison with the spectrum of ligands and the spectrum of prepared complexes. However, Table (3) shows the most important oscillatory vibrations of the important bonds in the ligands and the prepared complexes. Vibration ν of the group (C = N) is noted in the metal ions complexes with the prepared ligand; that displacements towards the higher or lower vibrations indicate the compatibility of the ligand with the metal ions. It ranged from 1609 cm^{-1} to 1624 cm^{-1} [12]. M-N vibrations appear in all the prepared complexes between 467 cm^{-1} and 441 cm^{-1} which appear as a weak or medium and sometimes strong [13,14].

TABLE 3. Characteristic of bands of IR of ligands and their metal complexes (cm^{-1})

Compounds	$\nu(\text{O-H});$ COOH	$\nu(\text{C-H});$ aromatic	$\nu(\text{C-H});$ aliphatic	$\nu(\text{-NH})$	$\nu(\text{C=O});$ COOH	vas(COO-)	$\nu(\text{C=N})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
$\text{C}_{35}\text{H}_{36}\text{F}_2\text{N}_8\text{O}_4\text{S}$ (LT)	3390	3156	2927	3289	1732	-	1624	-	-
$\text{C}_{35}\text{H}_{36}\text{ClCrF}_2\text{N}_8\text{O}_5\text{S}$ [Cr (LT)(H_2O) Cl]	3429	3179	2942	3255	-	787	1619	467	628
$\text{C}_{35}\text{H}_{36}\text{ClCoF}_2\text{N}_8\text{O}_5\text{S}$ [Co(LT)(H_2O) ₂]	3432	3183	2938	3267	-	782	1617	462	668
$\text{C}_{35}\text{H}_{36}\text{F}_2\text{MnN}_8\text{O}_5\text{S}$ [Mn (LT)(H_2O) ₂]	3428	3140	2927	3274	-	785	1612	441	656
$\text{C}_{35}\text{H}_{36}\text{ClF}_2\text{FeN}_8\text{O}_5\text{S}$ [Fe(LT)(H_2O) Cl]	3421	3187	2935	3188	-	785	1615	462	632
$\text{C}_{35}\text{H}_{36}\text{CuF}_2\text{N}_8\text{O}_5\text{S}$ [Cu(LT)(H_2O) ₂]	3424	3182	2935	3262	-	786	1614	458	628
$\text{C}_{35}\text{H}_{36}\text{F}_2\text{N}_8\text{O}_5\text{SZn}$ [Zn (LT)(H_2O) ₂]	3392	3177	2923	3269	-	788	1609	449	622
$\text{C}_{35}\text{H}_{36}\text{F}_2\text{N}_8\text{NiO}_5\text{S}$ [Ni(LT)(H_2O) ₂]	3417	3151	2935	3278	-	784	1611	466	623

UV-Visible Spectrum

The UV-visible spectrum has been measured in a dimethyl sulfoxide solvent (DMSO) at a concentration of 10^{-3} molar using a cell diameter 1cm at the room temperature. In this study, the displacement of the two absorption bands was shown where there was a clear change towards higher values and an increase in wavelength in the range from 15-35 nm, for the transition ($\pi \rightarrow \pi^*$) and up to 20-40 nm for the transition ($n \rightarrow \pi^*$) [15,16]. The increase in the intensity of the absorption beam due to the formation of the symmetric complex was explained. When interacting with metallic ions, the formation of symmetric complexes must be accompanied by often forming the coloured complexes with sharp and distinct absorption bands that differ from the ligand band either in a site, intensity or both.

Consideration was given to the type of the used ion for the salt, where chlorides were exclusively selected, as they are likely weighted [17]. On the other hand, the rest negative ions such as nitrates, sulphate, acetate or others, as well as the solutions are clear and free of sediments or the suspended particles which cause errors Table 4 shows UV – vis spectra of the free ligand and complexes in 10^{-3} M DMSO

TABLE 4. UV–vis spectra of the free ligand and complexes in 10^{-3} M DMSO

The Complex	$\lambda(\text{nm})$	$\epsilon(\text{cm}^{-1})$	ABS	$\epsilon \text{ L.mol}^{-1}\text{cm}^{-1}$	Assignment	Suggested Formula
(LT)	265	37735	1.150	1150	$\pi \rightarrow \pi^*$	
	315	31746	1.271	1271	$n \rightarrow \pi^*$	
	357	28011	0.980	980	$n \rightarrow \pi^*$	
	420	23809	0.460	490	$n \rightarrow \pi^*$	
[Cr (LT)(H ₂ O) Cl]	266	37593.9	1.352	1352	L.F	Octahedral
	314	31847.1	1.585	1585	L.F	
	346	28901.7	1.659	1659	L.F	
	415	24096.3	0.248	248	L.F	
	535	18691.5	0.064	64	${}^4A_{1g} \rightarrow {}^4T_{1g}(P)$	
	645	15503.8	0.056	56	${}^4A_{1g} \rightarrow {}^4T_{1g}(F)$	
	905	11049.7	0.008	8	${}^4A_{1g} \rightarrow {}^4T_{2g}(F)$	
[Co(LT)(H ₂ O) ₂]	266	37593	1.327	1327	L.F	Octahedral
	312	32051.2	1.851	1851	L.F	
	357	28011.2	1.423	1423	L.F + C.T	
	485	20618.5	2.167	2167	${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$	
	677	14771.0	0.306	306	${}^4T_{1g} \rightarrow {}^4A_{2g}(F)$	
					${}^4T_{1g} \rightarrow {}^4T_{2g}(F)$	
[Ni(LT)(H ₂ O) ₂]	266	37593.9	1.368	1368	L.F	Octahedral
	312	32051.2	1.866	1866	L.F	
	348	28735.6	1.788	1788	L.F	
	357	28011.2	1.411	1411	L.F + C.T	
	410	24390.2	0.186	186	${}^3A_{1g} \rightarrow {}^3T_{2g}(P)$	
	797	12547.0	0.102	102	${}^3A_{1g} \rightarrow {}^3T_{1g}$	
					${}^3A_{1g} \rightarrow {}^3T_{2g}$	
[Cu(LT)(H ₂ O) ₂]	269	37174.7	1.760	1760	L.F	Octahedral
	316	31645.5	1.587	1587	L.F	
	360	27777.7	1.508	1508	L.F	
	405	24691.3	2.016	2016	C.T	
	953	10493.1	0.528	528	${}^2E_g \rightarrow {}^2T_{2g}D$	
[Fe(LT)(H ₂ O) Cl]	269	37174.7	1.731	1731	L.F	Octahedral
	313	31948.8	2.031	2031	L.F	
	345	28985.5	1.909	1909	L.F	
	390	25461.0	0.608	608	L.F	
	515	19417.4	0.206	206	${}^5T_{2g} \rightarrow {}^5E_gD$	
[Mn (LT)(H ₂ O) ₂]	265	37735.8	1.266	1266	L.F	Octahedral
	313	31948.8	1.781	1781	L.F	
	345	28985.5	1.610	1610	L.F + C.T	
	357	28011.2	1.368	1368	L.F	
	696	14367.8	0.216	216	${}^6A_{1g} \rightarrow {}^4T_{1g}G$	
	850	11764.7	0.068	68	${}^6A_{1g} \rightarrow {}^4T_{1g}D$	
[Zn (LT)(H ₂ O) ₂]	269	37174.7	1.031	1031	L.F	Octahedral
	316	31645.5	1.087	1087	L.F	
	405	24691.3	1.316	1316	C.T	

Thermal Studies

The Thermo Gravimetric Analysis of Complexes has been diagnosed by TGA Curve. This technique is used for measuring the mass change in terms of the temperature when the material is controlled by a heating program at a given time called the resulting curve. Some information can be obtained from the weight curve associated with thermal stability, reaction speed, the chemical composition of the model, thermal stability of products and

determination of the type of reaction whether emitter or endothermic. Table 4 shows the results that correspond to the proposed general formula of the complexes. This table also shows information of each stage of pyrolysis [18] compounds metal complexes were examined by thermo gravimetric analysis in the temperature range of 30–600 °C obtained results from the TG curves for all these compounds are given in Table 5. The decomposition was complete at 596°C for all the complexes shows that the complexes are more thermally stable were are given in Table (3.6) and their thermographs are shown in Figures (6-7) respectively. The following results were obtained The first stage which took place at temperature range of (57-244)°C. includes departure of the Lattice water molecules and methanol . The following stage at temperature range of (161-374)°C includes departure of the coordinated water molecules and chloride ions at temperatures depending on bond energies resulted from coordination of ligand atoms The third stage (237-577) involved the departure of some other fragments of the ligands It loses weight 82.366% and 91.47% respectively, after which it stays stable until it decomposes Co and Cr are complex until the temperature reaches 596 ° C.

TABLE 5 TGA data of the complexes Co(II) and Cr(III)

Complexes.	Step	TGA				Reaction	Total mass loss%
		T _i /°C	T _f /°C	T _{DTG} max	Weight mass loss% calc (found)		
[Co(LT)(H ₂ O) ₂]	1	57.6	161.5	93.6	10.022(11.412)	-H ₂ O, CO ₂ Cl	82.336 (82.556)
	2	161.5	237.1	209.6	11.068(10.665)	C ₆ H ₄ N	
	3	237.1	393.4	318.6	24.599(25.411)	C ₁₃ H ₈ N ₄ S	
	4	393.4	596.5	467.5	36.647(35.068)	C ₁₅ H ₂₂ N ₃ OF ₂ CoO	
The practical lost weight% (82.556) and the remaining% (17.444) Theoretically lost weight% (82.336) and the remaining% (17.664)							
[Cr (LT)(H ₂ O) Cl]	1	76.5	244.1	175.4	38.203(35.960)	-H ₂ O, C ₁₃ H ₈ N ₄ SF ₂	91.475 (91.733)
	2	244.1	374.6	300	15.566(16.887)	-C ₆ H ₄ NCl	
	3	374.6	577.5	476.1	37.706(38.886)	-C ₁₆ H ₂₂ N ₃ O ₃ -CrO ⁺	
The practical lost weight (91.733%) and the remaining% (8.267) Theoretically lost weight% (91.475) and the remaining% (8.525)							

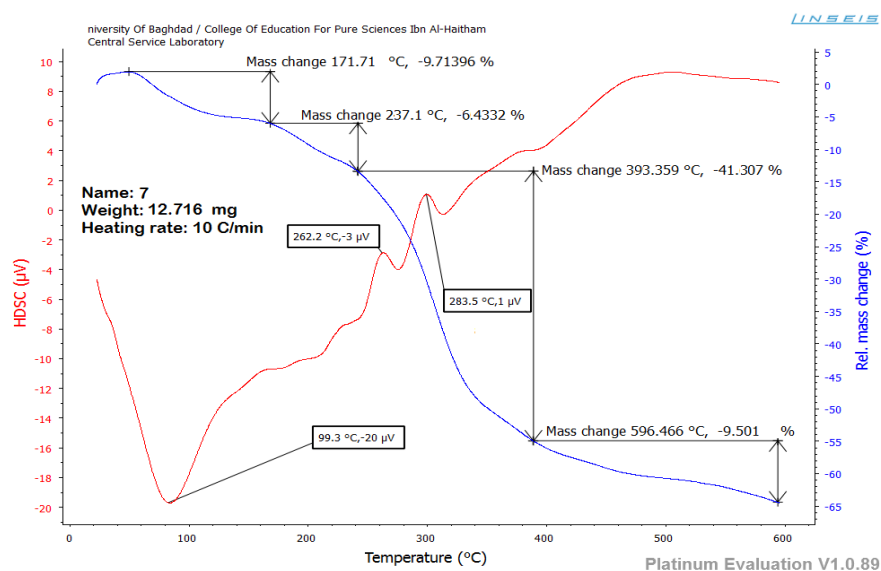


FIGURE 6. Complex gravimetric thermal decomposition curve [Co (LT) (H₂O) Cl]

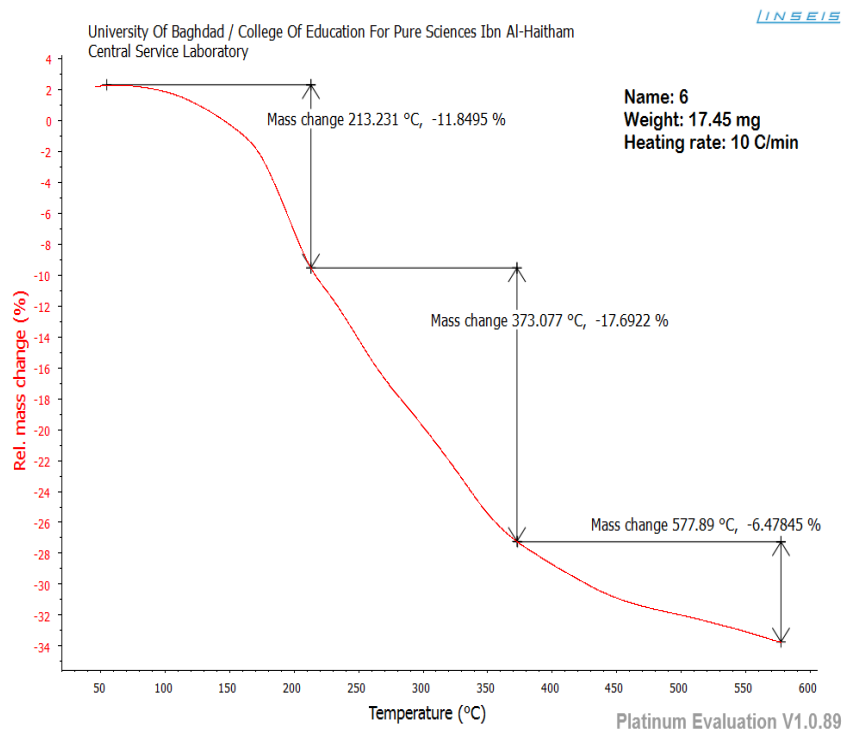


FIGURE 7. Complex gravimetric thermal decomposition curve [Cr (LT)(H₂O) Cl]

The Biological Activity

A few special treatments for infectious diseases were discovered. All of which were from natural sources until 1915. Ehrlich Paul discovered many useful drugs which were added to the branch of medicine called chemotherapy, which includes the treatment of bacterial diseases by chemicals. The factor that destroys microorganisms without affecting receptor tissue [19, 20]. Two bacterial species have been applied in this study: Gram-positive and Gram-negative bacteria (*Pseudomonas*, *Staphylococcus aureus*, *Bacillus* and *Escherichia coli*). The influence of the ligand and most prepared complexes against two species of bacteria was studied. However, DMSO was used as a solvent in this test. Most of the ligands are effective, direct bacteria are medium that is used, and most complexes ranged from medium to high. The surrounding cell allows only fat-soluble substances which make fat solubility as the important factor that controls antibacterial activity. Therefore, the complex spreads faster through the cell membrane and in the lipid layers of the microorganism wall and then enters and destroys the bacterial cell [21]. On other hand, transitional metals also have an antimicrobial effect [22,23].

TABLE 6. Zone of inhibition of the ligand and the synthesized complexes (mm or %)

The compounds	Bacteria			
	<i>Staphylococcus aureus</i>	<i>Pseudomonas</i>	<i>Bacillus</i>	<i>Escherichia coli</i>
Ciprofloxacin	24	19	29	24
(LT)	31	28	33	27
[Ni(LT)(H ₂ O) ₂]	38	35	40	33
[Cu(LT)(H ₂ O) ₂]	41	35	45	39
[Cr (LT)(H ₂ O) Cl]	43	38	51	44
[Co(LT)(H ₂ O) ₂]	39	43	42	38
[Mn (LT)(H ₂ O) ₂]	33	29	37	46
[Fe(LT)(H ₂ O) Cl]	39	34	41	38
[Zn (LT)(H ₂ O) ₂]	41	38	35	34
Control (DMSO)	0	0	0	0
thiourea	2	0	0	3

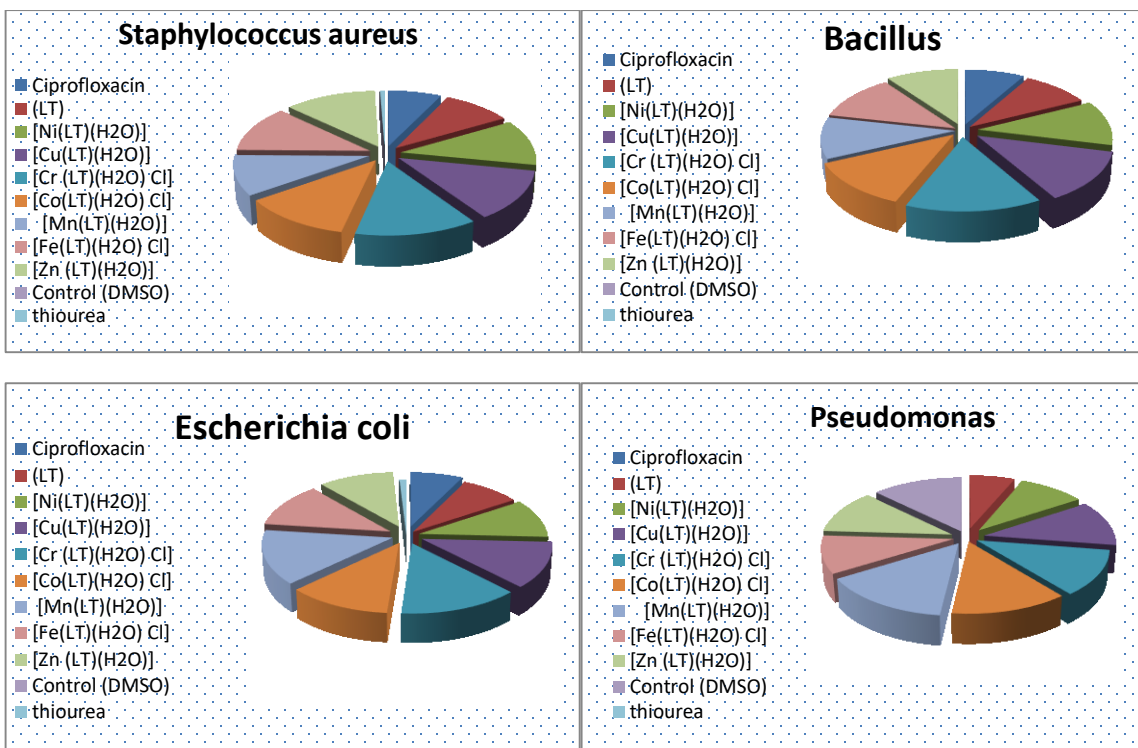


FIGURE 8. The Biological Activity.

CONCLUSION

New Schiff base complexes of Zn (II), Cu (II), Co (II), Fe (III), Cr (III), Ni (II), and Mn (II) were prepared. Schiff base (LT) is derived from the interaction of ciprofloxacin with thiourea. It has been prepared by tetradentate ligand having N and O donor atoms. All complexes are partially dissolved in water but fully dissolved in solvents of DMF, DMSO, and non-electrolyte solvents. The geometry of the prepared complexes was proposed based on the measurement of UV-Vis spectrum, FT-IR, and molar conductivity, magnetic sensitivity. All octahedral complexes except complexes of Zn (II) and Cu (II) were found to be tetrahedral. Biological efficacy was conducted toward four species of bacteria including *Staphylococcus aureus* and *Pseudomonas*, *Bacillus*, *Escherichia coli*. The biological efficacy of the complexes was greater than ones of the ligand.

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