Journal of Basic and Environnemental Sciences, 6 (2019) 139-148

ISSN
Online: 2356-6388
Print: 2536-9202

Research paper Open Access

Transition Metal Complexes of Biologically Active Ligands; Preparation, Characterization and Biological Evaluation

Moustafa E. Moustafa, Helmy E. Megahed and Doaa S. Yousef
Chemistry Department, Faculty of Science, Benha University, Benha, Egypt.

Abstract

The mixed ligands complexes formed between the biologically active ligands; N-Acetylcysteine (NAC) and 2-Acetylthiophene (AcTH) (secondary ligands), levofloxacin (Levo) (primary ligand) and Mn^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+} and Cu^{2+} were prepared and their chemical structures were studied and characterized by elemental analysis, molar conductivity, FT-IR, UV-Vis spectroscopy, magnetic moments, and thermogravimetric (TG&DTA) analysis. Spectral and elemental analysis data showed that both levofloxacin drug acts as monovalent bidentate coordinating to the metal ion via OO fashion while the secondary ligands act as neutral bidentate coordinating through OS fashion with octahedral geometry in all cases. The antimicrobial activity of the mixed ligand complexes has been studied by screening against some gram positive and gram-negative bacteria, where it was found that they have enhanced activity. The antitumor activities of some selected complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug.

Keyword: mixed ligand complexes, levofloxacin, N-Acetylcysteine, 2- Acetyl-thiophene, antimicrobial, antitumor activity.

Received; 15 Oct 2018, Revised form; 28 Dec. 2018, Accepted; 28 Dec. 2018, Available online 1 April 2019

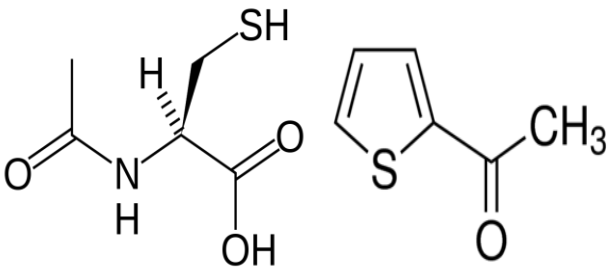
1. Introduction

Many mixed ligand metal complexes have provoked wide interest because they possess a diverse spectrum of biological and pharmaceutical activities, including antitumor, anti-HIV, antioxidative, antifungal, and antibacterial activities [1-15]. In view of the wide biological activities exhibited by drug-M-ligand complexes, it is meaningful to extend these studies on mixed ligands metal complexes containing levofloxacin-biologically active ligands. One of the major applications of the transition metal complexes is their medical testing as antibacterial and antitumor agents aiming to discover an effective and safe therapeutic regimen for the treatment of bacterial infections and cancers [16]. So, this paper reports the synthesis, characterization, and biological studies of mixed ligand transition metal complexes prepared from levofloxacin as a primary ligand and N-Acetylcysteine (NAC) and 2- Acetylthiophene (AcTH) as secondary ligands. The molecular structures were cofermed and the antibacterial and antitumor activities of some complexes were screened.

2- Experimental

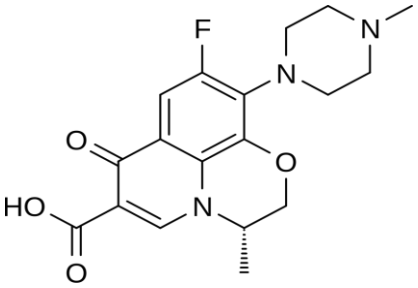
2.1. Materials

All reagents used in the present study were of the highest quality (Merck, Aldrich and Sigma chemicals). N-Acetylcysteine (NAC) and 2- Acetylthiophene (AcTH), as secondary ligands were used without further purification. Levofloxacin was obtained from Egyptian Company for Chemicals and Pharmaceuticals. Freshly bidistilled water was used whenever water is necessary. The ligands under study have the following chemical structure:



N-Acetylcysteine

2- Acetylthiophene



Levofloxacin

2.2. Preparation of the mixed ligands solid complexes

Mixed ligand Mn^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+} or Cu^{2+} complexes were prepared from hydrated metal chloride, Levofloxacin as primary ligand, N-acetylcysteine and 2-acetyl-thiophene as secondary ligands.

Aqueous solution of 1.00 mmol of metal salts was added with stirring to an ethanolic solution of 1.00 mmol of each of the secondary ligand and the mixture was refluxed for ≈ 3 hours. To the above mixture, an ethanolic solution of Levofloxacin (1.00mmol), as primary ligand, was added. The mixture (1:1:1 molar proportion) was again refluxed in a water bath for 5 hours. The mixture was then cooled to room temperature, and the solid complexes so formed were filtered off and washed with distilled water followed by ethanol and dried under vacuum.

2.3. Physical measurements

Elemental analysis; (C, H and N) were carried out at the Regional Center for Mycology and Biotecnology, Al-Azhar University, Cairo, Egypt. Metal ion contents (Mn, Fe, Co, Ni and Cu) were determined by EDTA titration under the appropriate conditions **[17]**. Infrared spectra were recorded as KBr disc technique using FT–IR spectrometer Model Nicolet is10-thermo-scientific within the wavenumber range 4000 – 400 cm^{-1}, at Faculty of Science, Benha University. Thermogravimetric analyses were carried out using Shimadzu TGA – 50H thermal analyzer within the range 25ºC–800ºC. All measurements were done under nitrogen atmosphere at heating rate of 10ºC per minute. The electronic absorption spectra in solution (DMF as a solvent) and in solid state (Nujol mull technique) were recorded on Jasco V-530 (UV-Vis) double beam spectrophotometer (Japan) with scanning speed 400 nm/min and band width 2.0 nm using 10 mm matched quartz cell at room temperature in the range 800 – 200 nm. The magnetic moment values were measured at room temperature using a Sherwood scientific magnetic susceptibility balance. Molar conductivities of the complexes (10^{-3} M) in DMF were obtained using a conductivity bridge YSI model 32.

2.4. Antimicrobial Screening

The antibacterial activity of some selected mixed ligands complexes toward four bacterial strains (two gram positive: *Streptococcus pyogenes* and *Staphylococcus epidermidis* and two gram-negative bacteria; *Proteus vulgaris* and *Klebsiella pneumonia*) was evaluated by agar well diffusion method [18]. The tested compounds were dissolved in DMF which have no inhibition activity to get concentration of 100 mg/ml. A hot nutrient agar solution (20 ml) was poured into the sterilized petri dishes and allowed to attain room temperature. The seed layer medium was melted and cooled to ≈ 45ºC with gentle shaking. The previously grown subculture was added to the seed layer medium aseptically and mixed well. It was immediately raked into the petri dishes and allowed to attain room temperature. Then wells were made with the sterile cork porer and to these wells, 50 µl in concentration of 100 mgl/ml of the tested compound was added and the plates were allowed to cool for one hour to facilitate the diffusion. The plates were incubated at 37ºC for 24 hours. Antibacterial activity of the complexes was evaluated by measuring the diameter of zone of inhibition in mm. The medium with DMF as solvent was used as a negative control whereas media with Ampicillin were screened separately for its standard antibacterial activity.

2.5. Antitumor activity assay

The antitumor activity assay toward Human hepatocellular carcinoma (HepG2) cell line was studied according to reported technique **[19, 20]**. All measurements were carried out at the Regional Center for Mycology and Biotecnology, Al-Azhar University, Cairo, Egypt. The tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning R 96-well tissue culture plates, and then incubated for 24 hrs. The tested compounds were then added into 96-well plates (six triplicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubation for 24 hr, the number of viable cells was determined by the MTT test [19, 20]. The optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc. USA) to determine the number of viable cells and the percentage of viability was calculated as

$$(1 - \frac{ODt}{ODc}) \text{ x100}$$

Where ODt is the mean optical density of the wells treated with the tested samples and ODc is the mean optical density of the untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effect in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration.

3. Results and discussion

A- Structure Elucidation of the Mixed Ligands Metal Complexes:

1- Elemental analysis and molar conductivity

All the complexes are colored, stable in air and have high melting points. They are freely soluble in DMSO and DMF but sparingly soluble in other common organic solvents. The metal complexes were characterized by elemental analysis, molar conductivities, thermal analysis, IR and UV-Vis spectra. Results of elemental analysis (Tables 1 and 2) are in good agreement with the calculated values of the proposed formulae. The values reveal that the primary ligand: metal ion: secondary ligand ratio is 1:1:1.

The chemical composition (C, H and N), percent of metal ion, chloride ion and water molecules in the solid complexes can be determined by elemental analysis. The determination of chloride ion content was performed by Volhard's method, the metal ion concentration by EDTA titration under appropriate conditions [17] and the number

of water molecules attached to the metal chelates was determined by the dehydration method. Based on the data given, the corresponding probable constitutional formula for the different complexes are suggested and given in Table (1). The molar conductivities of the studied mixed ligands complexes are measured in solutions of DMF. The molar conductance (Λ_m); $ohm^{-1}cm^2mol^{-1}$ is given by the relation: $\Lambda_m = K/C$, where K is the cell constant (ohm^{-1}) and C is the concentration of the complex in mole/l. The molar conductance values are 33.12 and 35.47 $ohm^{-1}cm^2mol^{-1}$ for Fe^{3+} complexes indicating the ionic nature of these complex with number of ions equal three. On the other hand, the values of Mn^{2+}, Ni^{2+} and Cu^{2+} are within the range 18.22 - 23.55 $ohm^{-1}cm^2mol^{-1}$ indicating their ionic nature with number of ions equal two. The presence of the anion (Cl^-) outside the coordination sphere is confirmed by the precipitation of Cl^- as AgCl by the addition of $AgNO_3$ solution to the solubilized chelates in DMF.

Table (1): Elemental analysis and molar conductivity of Levofloxacin – 2-acetylthiophene mixed metal complexes

Compound	M.Wt	%C	%H	%N	%M	Λ_m
$C_{24}H_{26}O_5N_3SF$	487.15	59.12 (56.07)	5.38 (5.44)	8.62 (8.44)	……..	……
$C_{24}H_{26}O_5N_3SF(H_2O)_2MnCl$	613.086	46.98 (47.01)	4.93 (5.11)	6.85 (6.43)	8.96 (9.11)	19.05
$C_{24}H_{26}O_5N_3SF(H_2O)_2FeCl_2$	649.051	44.37 (44.21)	4.66 (4.79)	6.47 (6.32)	8.62 (8.48)	33.12
$C_{24}H_{26}O_5N_3SF(H_2O)_2CoCl$	617.082	46.65 (47.01)	4.89 (4.66)	6.80 (7.02)	9.54 (9.34)	18.22
$C_{24}H_{26}O_5N_3SF(H_2O)_2NiCl$	616.083	46.75 (46.20)	4.91 (5.20)	6.82 (6.55)	9.40 (9.27)	20.11
$C_{24}H_{26}O_5N_3SF(H_2O)_2CuCl$	622.58	46.80 (46.22)	4.86 (4.33)	6.75 (6.37)	10.21 (10.05)	19.61

Table (2): Elemental analysis and molar conductivity of Levofloxacin – 2-acetylcysteine mixed metal complexes

Compound	M.Wt	%C	%H	%N	%M	Λ_m
$C_{23}H_{29}N_4O_7FS$	524.174	52.66 (51.93)	5.57 (5.33)	10.68 (10.11)	…..	…..
$C_{23}H_{29}N_4O_7FSMn(H_2O)_2Cl$	650.102	42.44 (42.11)	5.11 (5.07)	8.61 (8.35)	8.44 (8.08)	19.22
$C_{23}H_{29}N_4O_7FSFe(H_2O)_2Cl_2$	686.068	40.26 (39.68)	4.85 (5.06)	8.16 (8.34)	8.15 (8.33)	35.47
$C_{23}H_{29}N_4O_7FCo(H_2O)_2Cl$	654.98	42.18 (42.55)	5.08 (5.21)	8.55 (8.44)	9.00 (8.75)	20.44
$C_{23}H_{29}N_4O_7FSNi(H_2O)_2Cl$	653.099	42.24 (42.67)	5.09 (5.04)	8.58 (8.44)	8.87 (8.74)	18.55
$C_{23}H_{29}N_4O_7FCu(H_2O)_2Cl$	626.122	44.02 (43.96)	5.30 (5.22)	8.93 (8.75)	10.13 (10.05)	19.36

2-Thermal analysis

Thermal methods of analysis open up a new possibility for the investigation of metal complexes. It is taken, in addition to elemental analysis, as a tool to confirm the chemical structure of the complexes. The degradation steps of the samples can be followed up and the different degradation fragments can be estimated in the light of molecular structure of the complexes as given in Table (3), from which it is shown that the complexes under study are thermally degraded in, more or less, three main steps.

1- The first step, maximally ended at 189°C, is due to the removal of either physically adsorbed or

coordinated water molecules attached to the solid complexes in addition to some gases produced from the pre-decomposition of the complexes such as HCl, HF, N_2 or CO_2.

2- The second degradation step, within the temperature range 225ºC - 329ºC is due to the thermal decomposition of either N-acetylcysteine (NAC) or 2- Acetylthiophene (AcTH) acting as secondary ligands. The experimental weight loss of this step agrees well with the calculated values of the degraded fragment (c.f. Table 3).

3- The final step, represented by strong endothermic peaks centered at ≈ 550ºC, is due to the decomposition of the levofloxacin moiety of the complexes leading to the formation of metal oxides as final products from which the % of metal ion is calculated as given in Table (3).

Table (3): Thermogravimetric data for levofloxacin – M – NAC, AcTH complexes

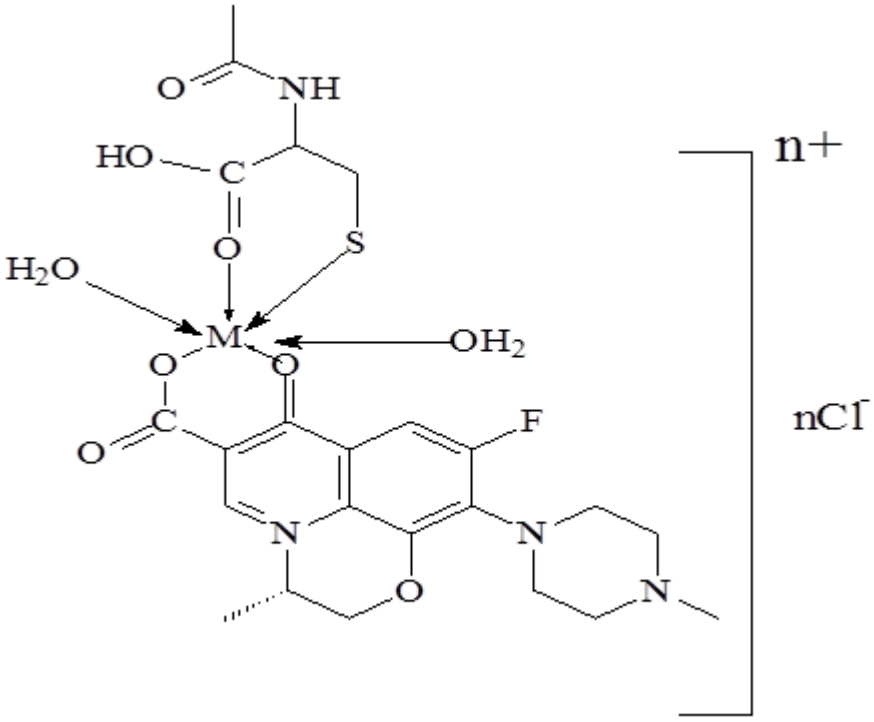
Complex	Temp.(ºC)	Weight loss %	Assignment
Lev-Ni-NAC	164.5	8.31 (9.57)	Removal of coordinated H_2O molecule, HCl, HF
	255.8	40.23(39.99)	Beginning of decomposition of NAC
	530.54	34.16(47.55)	Decomposition of Levofloxacin Moiety leading to NiO_2; 17.30 %. Ni% = 13.59 (15.22)
Lev-Cu-NAC	70.55	7.80	Removal of hydrated H_2O molecule.
	188.4	33.33(39.74)	Beginning of decomposition of NAC, HCl gas
	524.23	42.39(44.76)	Decomposition of Levofloxacin Moiety leading to Cu_2O; 149 %. Cu% = 13.17 (14.44)
Lev-Fe-NAC	92.12	----	Removal of hydrated H_2O molecules.
	216.08	Composite 3 steps	Beginning of decomposition of NAC, HCl, HF and CO_2 gases Decomposition of Levofloxacin Moiety leading to Fe_2O_3; 20.73
	495.45	---	%. Fe% = 17.64 (16.41)
Lev-Ni-AcTH	88.56	----	Removal of hydrated H_2O molecules.
	242.60	45.30(47.22)	Beginning of decomposition of AcTH, HCl, CO_2 gases
	530.11		Decomposition of Levofloxacin Moiety leading to NiO; 10.64 %. Ni% = 8.36 (7.73)
Lev-Zr-AcTH	82.59	----	Removal of hydrated H_2O molecules.
	133.61	31.25 (31.39)	Beginning of decomposition of AcTH, HCl, HF and CO_2 gases
		---	Decomposition of Levofloxacin Moiety leading to Fe_2O_3; 18.27
	463.73		%. Fe% = 15.55 (11.55)
Lev-Cu-AcTH	124.26	----	Removal of hydrated HCl, CO_2, H_2O.
	2255.37	31.25(31.29)	Beginning of decomposition of AcTH.
	533.06	48.44(49.10)	Decomposition of Levofloxacin Moiety leading to CuO; 9.86 %. Cu% = 7.87 (8.64)

Values between parentheses are theoretical values.

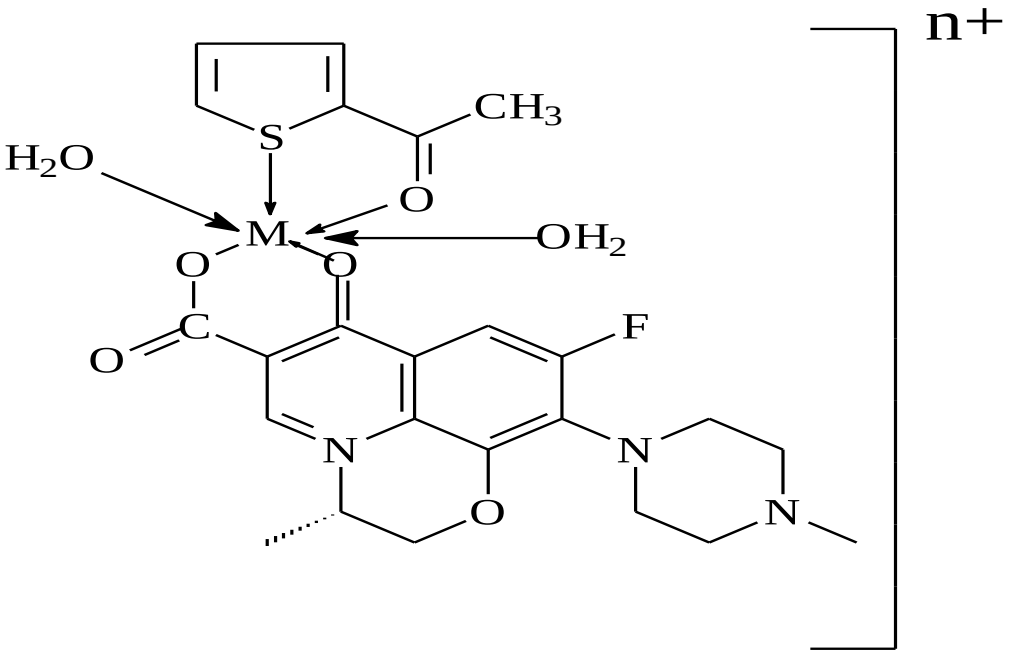
4- IR Spectra

Fundamental IR spectral bands of levofloxacin, *N*-acetylcysteine and 2-acetylthiophene compounds were examined and compared to those of mixed ligands complexes. The IR spectrum of free levofloxacin is characterized mainly by the strong bands at 3424, 1671, 1453, 1164 and 982 cm^{-1} due to stretching vibration of V_{OH}, $V_{C=O}$, V_{C-S}, V_{COO^-} and δOH, respectively (*c.f.* Table 4). On the other hand, the spectra of the free *N*-acetylcysteine and 2-acetylthiophene compounds acting as secondary ligands are characterized by the bands within the ranges 3424-3376 cm^{-1}, 1671-1625 cm^{-1}, 1446-1427 cm^{-1}, 1158-1155 cm^{-1} and 1092-975 cm^{-1} due to V_{OH}, $V_{C=O}$, V_{C-S}, V_{COO-} and V_{NH}, respectively. On complexation, the stretching vibration bands due to C=O and C-S groups of both *N*-acetylcysteine and 2-acetylthiophene are shifted to lower wavenumber indicating that they coordinate to metal ions through OS fashion acting as neutral bi-dentate ligands. At the same time, the stretching vibrations of OH and COO^- groups of the levofloxacin moiety also suffer a shift to lower wavenumber indicating the contribution of these groups in complex formation via OO fashion and shows that levofloxacin acts as monovalent monodentate ligand. The far infrared region of the spectra of the metal chelates shows new sets of bands within the region 600 – 400 cm^{-1} which are due to the stretching vibrations of M – O and M – S bonds.

Elemental analysis confirmed the formation of Mn2+, Fe3+, Co2+, Ni2+ and Cu2+ mixed ligand complexes with levofloxacin, *N*-acetylcysteine and 2-acetylthiophene with stoichiometric ratio 1:1:1, accordingly the mode of bonding of such complexes can be represented as follows:



Levofloxacine – 2-acetylcysteine complex
M = Mn^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+} or Cu^{2+}; n =2 for Fe^{3+} and =1 for M^{2+}



Levofloxacine – 2-acetylthiophene complex
M = Mn^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+} or Cu^{2+}; n =2 for Fe^{3+} and =1 for M^{2+}

Table (4): IR vibrational frequencies (cm^{-1}) of some function groups of levofloxacin – M – NAC, AcTH mixed complexes

Compound	V_{OH}	$V_{C=O}$	V_{COO^-}	V_{C-S}	V_{M-S}	V_{M-O}
Levofloxacin	3262	1725	1164	1082	---	---
NAC	3424	1733	---	1066	---	---
AcTH	----	1752	…..	1080		
Levo-Mn-NAC	3271	1715	1160	1068	710	565
Levo-Fe-NAC	3355	1784	1147	1059	755	565
Levo-Ni-NAC	3320	1786	1160	1050	775	580
Levo-Co-NAC	3388	1713	1152	1055	720	545
Levo-Cu-NAC	3241	1710	1160	1075	725	550
Levo-Mn-AcTH	3266	1685	1158	1080	735	550
Levo-Fe- AcTH	3309	1675	1156	1065	742	565
Levo-Ni- AcTH	3345	1558	1162	1072	725	560
Levo-Co- AcTH	3315	1635	1160	1065	738	550
Levo-Cu- AcTH	3285	1628	1158	1075	720	562

4- **Magnetic susceptibility**

Magnetic susceptibility measurements at room temperature show the paramagnetic nature for all the complexes. The μ_{eff} values, term symbols and ground state symbols are listed in Table (5). The magnetic susceptibility values of the Mn^{2+} complexes are 5.32 and 5.45 BM and those for Fe^{3+} complexes are 5.41 and 5.33 BM for Levo – NAC and AcTH complexes, respectively which support the high spin state of Mn(II) and Fe(III) ions in the complexes under study. The Ni(II) complexes show magnetic moment values of 2.82 and 2.79 BM for Levo-NAC-Ni^{2+} and Levo-AcTH-Ni^{2+} complexes respectively, indicating an octahedral environment around Ni(II) ion [21,22]. The observed magnetic moments for the Cu(II) complexes are 1.75 and 1.76 BM for the two complexes, respectively suggesting a distorted octahedral geometry for Cu(II) complex [23].

5- Electronic spectra

Electronic absorption spectra of the mixed ligands complexes were scanned in solution using DMF as solvent and in solid state using Nujol mull technique. The electronic absorption spectral data in Nujol mull (wavenumber; cm^{-1}) are given in Table (5). Inspection of the data obtained shows that:

1-The high spin Fe (III) complexes with d^5 configuration have the ground state $^6A_{1g}$ and all the d-d transitions are spin and laporte forbidden. The electronic spectra of Fe(III) complex display three bands at 95238.1, 10204.1 and 23809.9 cm^{-1} assignable to $^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $^6A_{1g}(S) \rightarrow {}^4T_{2g}(G)$ and $^6A_{1g}(S) \rightarrow {}^4E_g, {}^4A_{1g}(G)$ transitions, respectively, indicating that the complex possesses a high spin octahedral configuration [24].

2-Cobalt (II) complexes show three bands at 16666-16806 cm^{-1},14749-14925 cm^{-1} and 13679-13698 cm^{-1} which are due to d→d electronic transition of the types $^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$ (V_1), $^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$ (V_2) and $^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$ (V_3), respectively [25].

3-The electronic spectra of Ni (II) complexes show the three spin allowed bands at (31055-31250 cm^{-1}), (22222-22573 cm^{-1}) and (18155-18181 cm^{-1}) which are assigned to electronic transition type $^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$, $^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$ and $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}$, respectively.

4-Copper (II) complexes show two spin allowed transition bands within the range 22321– 22727 cm^{-1} and 18797– 19230 cm^{-1} due to the $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ and $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$ transitions, respectively. It was reported that Cu (II) complexes showed a broad asymmetric band in the region 20576 cm^{-1} expected for a d→d transition in an octahedral Cu (II) complex [26]**.** The broadness of the band could be attributed to the overlapping of several bands as a result of strong Jahn-Teller distortion expected in a d^9 ion. [27]**.**

Table (5): electronic absorption spectra and magnetic moment data of the mixed ligands complexes in Nujol mull.

Complex	wavenumber. (cm^{-1})	Assignment	Magnetic data		
			Term Symbol	Ground state	μ_{eff} (BM)
Levo-NAC-Fe^{3+}	30251.8 2553.5 17264.3	$^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $\rightarrow {}^4T_{2g}(G)$a $\rightarrow {}^4E_g$,	^6S	$^6A_{1g}(S)$	5.41
Levo-NAC-Co^{2+}	27444.8 24125.6 12994.6	$^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}(\nu_1)$ $\rightarrow {}^4T_{1g(P)}(\nu_2)$ $\rightarrow {}^4A_{2g(F)}(\nu_3)$	^4F	$^4T_{1g}$	4.09
Levo-NAC-Ni^{2+}	22283.0 16116.4 13552.8	$^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\nu_1)$ $\rightarrow {}^3T_{1g(F)}(\nu_2)$ $\rightarrow {}^3T_{1g(P)}(\nu_3)$	^3F	$^3A_{2g}$	2.82
Levo-NAC-Cu^{2+}	16863.4 14285.7 13123.4	 $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$	^2D	^2E_g	1.75
Levo- AcTH - Fe^{3+}	36442.5 27763.1 19957.3	$^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$, $\rightarrow {}^4T_{2g}(G)$a $\rightarrow {}^4E_g$,	^6S	$^6A_{1g}(S)$	5.33
Levo- AcTH - Co^{2+}	26887.2 24351.5 16648.2	$^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}(\nu_1)$ $\rightarrow {}^4T_{1g(P)}(\nu_2)$ $\rightarrow {}^4A_{2g(F)}(\nu_3)$	^4F	$^4T_{1g}$	4.09
Levo- AcTH - Ni^{2+}	20802.0 16553.7 14008.0	$^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\nu_1)$ $\rightarrow {}^3T_{1g(F)}(\nu_2)$ $\rightarrow {}^3T_{1g(P)}(\nu_3)$	^3F	$^3A_{2g}$	2.79
Levo- AcTH - Cu^{2+}	16559.2 14662.1 12688.2	$^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$	^2D	^2E_g	1.76

B- Biological activity

The antimicrobial activity of the mixed ligands – complexes is tested against representatives of gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and gram-negative bacteria (*Proteus vulgaris* and *Klebsiella pneumonia)*. Standard drug; Ampicillin and DMF solvent control were screened separately for their antibacterial activity. The antibacterial results, expressed as inhibition zone diameter (mm) and % activity index (relative to ampicillin), are given in Table (6) from which it is clear that the mixed ligands complexes show high activity against the tested organisms. Also, percent Activity Index data show that Cu (II) complexes have the highest activity followed by Ni (II), Co (II).

Increased activity of metal chelates had been explained by Overtone's concept [28] and the Tweedy's theory [29], according to which chelation reduces the polarity of the ligand due to partial sharing of its negative charge with the metal, favoring transportation of the complexes across the lipid layer of the cell membrane. The positive results suggested the very diffusion of the complexes into the bacterial cells and are able to kill the bacterium as indicated by the zones of inhibition of bacterial growth.

Table (6): Antibacterial activities of some mixed ligands complexes in terms of inhibition zone diameter (mm) and % activity index.

Complex	*Strept. Pyog.*		*Staph. Epid.*		*Prot. Vulgaris*		*Kleb. Pne.*	
	Inh. zone	% Ac.Ind	Inh. zone	% Ac.Ind	Inh. zone	% Ac.Ind	Inh. Zone	% Ac.Ind
Ampicillin	25	100	25	100	22	100	23	100
Levo	24	96	25	100	23	104.5	23	100
NAC	22	88	24	96	23	104.5	23	100
AcTH	23	92	21	84	20	90.9	21	91.3
Levo-NAC-Fe	21	84	20	80	19	86.3	20	86.9
Levo-NAC-Co	22	88	21	84	18	81.8	20	86.9
Levo-NAC-Ni	23	92	22	88	20	90.9	21	91.3
Levo-NAC-Cu	22	88	21	84	18	81.8	19	82.6
Levo-AcTH-Fe	23	92	20	80	18	81.8	16	69.5
Levo-AcTH-Mn	19	76	20	80	20	90.9	21	91.3
Levo-AcTH-Co	18	72	23	92	19	86.3	20	86.9
Levo-AcTH-Ni	20	80	19	84	19	86.3	20	86.9
Levo-AcTH-Cu	22	88	17	68	19	86.3	20	86.9

3.6. Antitumor activity

The cytotoxic activities of some selected mixed ligands complexes viz; Levo.–Fe-NAC, Levo.–Fe-AcTH, Levo.–Cu-NAC and Levo.–Cu-AcTH complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The relation between surviving cells and complex concentration is plotted to get the survival curve from which the 50% inhibitory concentration (IC_{50}) was estimated. The results are represented graphically in Fig (1) and the lethal concentrations (IC_{50}) values are listed in Table (7).

Table (7): Lethal concentration (IC_{50}) of the mixed ligands complexes on HEPG2 cell line.

Complex	IC_{50} (µg/ml)
Vinblastine	4.6
Levo.–Fe-NAC	15.6
Levo.–Fe-AcTH	33.1
Levo.–Cu-NAC	33.5
Levo.–Cu-AcTH	28.8

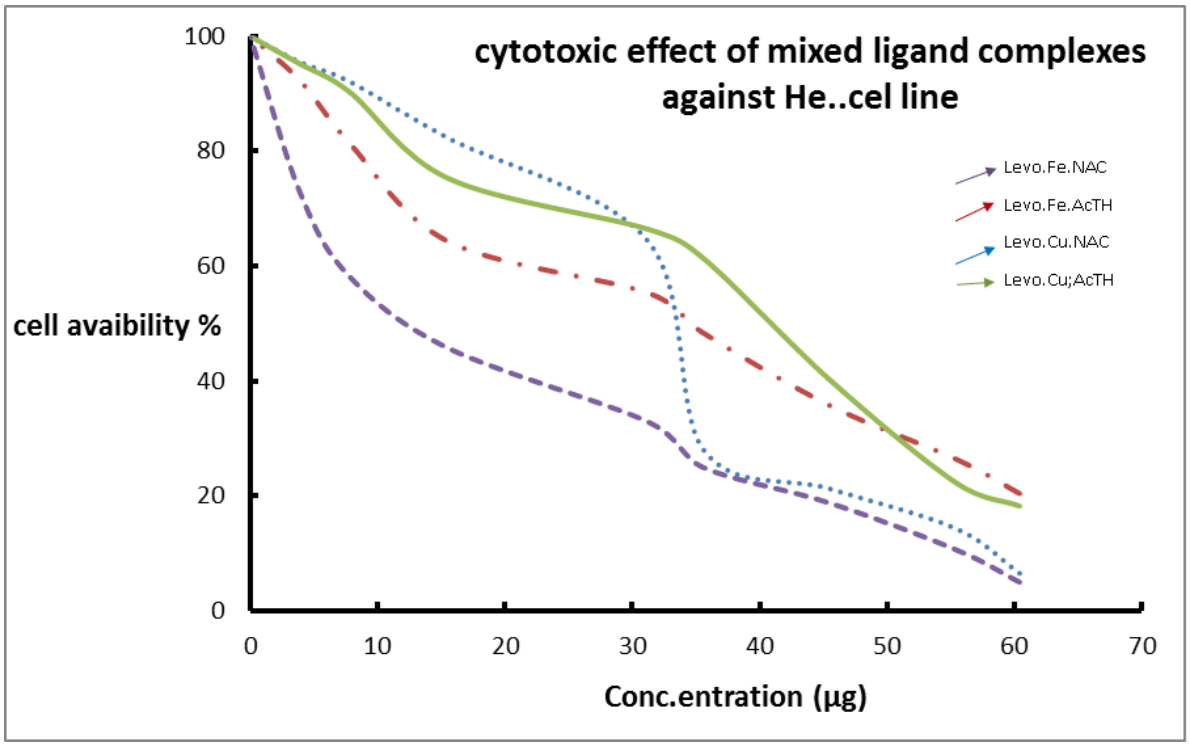


Fig (1): The relation between surviving cells and complex concentration.

Inspection of the cytotoxic data shows that mixed complexes of levofloxacin with AcTH are, in general, more effective than those with NAC complexes and the metal ions are arranged according to their cytotoxicity as Fe (III) > Cu (II). The enhanced activity of metal complexes may be attributed to the increase in conjugation in the ligand moiety takes place in complexation process [30]. Shier [30] suggested that compounds having IC_{50} values 10 – 25 µg/ml are considered to have weak cytotoxic activities, while those having intermediate values (ranging from 5 – 10 µg/ml) are classified as moderately active. On the other hand, compounds with IC_{50} values less than 5µg/ml are considered to be very active. Consequently, the mixed ligands complexes under study are considered to be ranged from weak to moderately active.

4. Conclusion

This work presents spectroscopic characterization and biological activity of mixed ligand complexes of Mn (II), Fe (III), Co (II), Ni (II) and Cu (II) with levofloxacin drug as primary ligand and two biologically active compounds; N-Acetylcysteine (NAC) and 2- Acetylthiophene (AcTH) as secondary ligands. The analytical and physicochemical data show satisfactory agreement with the proposed formulae confirming the structure of the prepared complexes. In brief, the results obtained can be summarized as follows:

1- The stoichiometric ratio of the complexes is (1:1:1) (Levofloxacin: M: secondary ligand) and the complexes are fairly stable in air and have non electrolytic nature.

2- Levofloxacin acts as monobasic bidentate ligand coordinated to metal ion through OO fashion whereas N-Acetylcysteine (NAC) and 2- Acetylthiophene (AcTH) coordinate via OS fashion acting also as neutral bidentate ligands.

3- The complexes show high antibacterial activity toward gram positive (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and gram negative (*Proteus vulgaris* and *Klebsiella pneumonia)* bacteria.

5- The cytotoxic activities of some selected mixed ligands complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The study reveals that the mixed ligands complexes are considered to be moderately active against HEPG2 cell line for Fe (III) complexes while Cu (II) complexes show weak activity with slightly high IC_{50}.

References

[1] D. A. Martins, R. L. R. Gouvea, G. S. Muniz, R. W. Sonia D. J. Batista, M. C. Soeiro and L. R. Teixeira; *Bioinorg. Chem. Appl.*, 5 (2016) 125

[2] A. E. Edith, E. E. Akporhonor and S. A. Osakwe; Chem. Mater. Res., 6 (6), (2014), 9.

[3] J. Devi , and N. Batra; Spectrochemica Acta (A) molec. Biomolec. Spect, 135, (2015), 710.

[4] H. Taghreed, F. A. Khalid, J. J. Amer and A. S. kindeel; Chem. Mater. Res., 3 (3), (2013), 126
[5] Z. Chen, B. Li, Y. Xie, R. Xiong, X. You and X. Feng; Inorg. Chem. Comm., 4, (7), (2001), 346–349
[6] S. Thakur, M. Farooqui and S.D. Naikwade; Intern. J. Pharm. Tech Res., 5, (4),(2013),508-1515.
[7] A. T. Abdelkarim, M. M. Al-Shomrani, A. M. Rayan and A. A. El-Sherif; J. Soln. Chem., 44,(8), (2015),1673-1704
[8] Ng C.Hee, S. M. Kong, Y. L. Tiong, M. J. Maah, N. Sukram, M. Ahmad and A. S. B. Khoo; Metallomics, ,6 (4), (2014), 892-906
[9] M. Sivasankaran Nair and S. Regupathy; J. Coord. Chem., 63, (2), (2010), 361-372.
[10] Patel MN , Chhasatia MR , Gandhi DS ; Bioorganic & Medicinal Chemistry, 17(15) (2009),5648-5655.
[11] G. J. Kharadi and K. D. Patel; App. Organometallic Chem., 23 (10), (2009), 391–397.
[12] R. M. N. Kumar, K. S. Prasad and N. Prasad; Chem., Sci.Trans, 4(3), (2014), 677-687.
[13] O. Golubev, T. Lönnberg and H. Lönnberg; Molecules, 19(10), (2014),16976- 16986.
[14] Y. Prashanthi, K. Kiranmai, K. S. kumar, V. k. Chityala and E. Shivaraj ; *Bioinorg. Chem. Appl.*, 3 (2012) 112.
[15] O. K. Amadi, I. E. Otuokere and C. F. Bartholomew; Res. J. Pharm. Dosage Forms and Tech., 7,(1), (2015), 59-68.
[16] M. E. Moustafa, Y. M. Issa, W. F. El-Hawary and M. Refaat; Spec. Lett., 32(5),(1999), 829-840.
[17] A. I. Vogel, "A Text Book of Quantitative Inorganic Analysis", Longmans, London, (1994).
[18] I. Ahmad and A. Z. Beg; J. Ethanopharmacology, 74, (2001), 113-123.
[19] T. Mosmann; J. Imn. Methods, 65, (1983), 55-63
[20] S. M. Gomha, S. M. Riyadh, E. A. Mahmmoud and M. M. Alaasser; Hetercycles, 91(6), (2015), 1227-1243.
[21] R.L. Prasad, A. Kushwaha, B.S. Gautam, *J. Coord. Chem*., 62, (2009), 2983.
[22] A. Earnshaw; Introduction to Magnetochemistry, Academic Press Inc Limited, London, 34, (1968).
[23] M. B. Halli, Ravindra. S. Malipatil, *Der Pharma Chemica.*, 3, (2011),146.
[24] Pranita U. G., Mandlik P. R. and Aswar A. S. , Ind. J. Pharm. Sci., 77(4), (2015) : 376-381.
[24] A. Earnshaw; Introduction to Magnetochemistry, Academic Press Inc Limited, London, 1968, 34.
[26] M. B. Halli, Ravindra. S. Malipatil, *Der Pharma Chemica.*, 2011, 3, 146.
[27] S. A. Shaker, Y. Farina, S. Mahmmod and M. Eskender; ARPN J. Eng. Appl. Sci., 4, (9), (2009),
[28] P. Kopel, Z. Travnicek, L. Kvitek, M. Biler, M. Pay licek, Z. Sindelar and J. Marek, Trans. Met. Chem, 26(3), (2001), 282 – 286.
[29] A. D. Garnovski and I. S. Vailchenko; Russ. Chem. Rev., 71, (2002), 943.
[33] W. T. Shier; Mammalian Cell Culture on $ 5 a day: a Laboratory Manual of Low Cost Methods. University of the Philippines, Los Banos, (1991).