Preparation, spectroscopic, thermal analyses and antibacterial studies of levofloxacin vanadium (V) complexes

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ABSTRACT

The new solid complexes $[VO(Lev)_2L]Cl$, where L= aniline (An), dimethylformamide (DMF), o-Tolidine (o-Tol) and pyridine (Py) have been synthesized. These complexes were characterized by melting point, magnetic studies, spectroscopic techniques (IR, UV-Vis, ^1H NMR), elemental analysis and thermal behavior of complexes also investigated. The results suggested that the molar ratio for all complexes is M:Lev:L=1:2:1 where levofloxacin acts as a bidentate via one of the oxygen atoms of the carboxylate group and the ring pyridone group and the complexes have the following formulae $[VO(Lev)_2An]Cl$, $[VO(Lev)_2DMF]Cl$ $[VO(Lev)_2o\text{-}Tol]Cl$ and $[VO(Lev)_2Py]Cl$. The activation energies, E^*, enthalpies, ΔH^*, entropies, ΔS^* and Gibbs free energies, ΔG^* of thermal decomposition reactions have been derived from thermo gravimetric (TGA) and differential thermo gravimetric (DTG) curves, using Coats-Redfern (CR) and Horowitz-Metzger (HM) methods. The antibacterial activity of these complexes has been evaluated against Gram-positive and Gram-negative bacteria and compared with the reference drug levofloxacin. The antibacterial activity of all complexes showed excellent activities against all microorganisms compared with free levofloxacin.

Keywords: Lev; ^1H NMR; IR; Kinetic parameters; Thermal analyses and Antibacterial activity.

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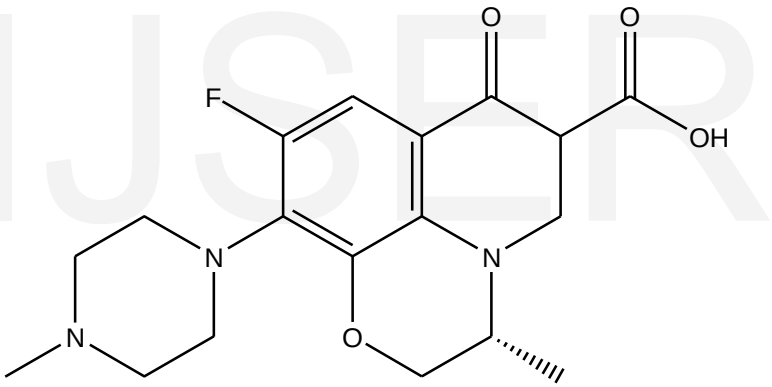
1. Introduction

Levofloxacin (Lev) or 6-fluoro-2,3-dihydro-3- methyl-10-(4-methyl-1-piperazinyl-7-oxo-7H pyrido [1,2,3-d]-1,4-benzoxazine)-3-carboxylic acid (Scheme 1), is a synthetic broad-spectrum antimicrobial fluoroquinolone active against both Gram-positive aerobic streptococcus pneumonia, staphylococcus aurous, Gram- negative Escherichia coli, moraxellacatharralis, haemophilus influenza, Klebsiella pneumonia, pseudomonas aeruginosa and intracellular pathogens responsible for atypical pneumonia **[1]**. It is used in the treatment of a wide range of bacterial infections includingacute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis. Along with other antibiotics it may be used to treattuberculosis, meningitis, or pelvic inflammatory disease. It is available by mouth or intravenously **[2, 3]**.



Scheme 1: Structure of levofloxacin (Lev).

Metals have played key role in the development of modern chemotherapy **[4-7]**. When dealing with the interaction between drugs and metal ions in living systems, a particular attention has been paid to the interaction of metal ions with antibiotics. The metal based drugs were also being used for the treatment of a variety of ailments viz. diabetes, rheumatoid arthritis, inflammatory and cardiovascular diseases **[8-10]**. Antibiotics that interact with metal ions constituted a new class of drugs

(fluoroquinolones) which has been widely used in medicine both for human beings and animals **[11, 12]**. Fluoroquinolones could participate in the formation of complexes in a number of ways **[13-17]**.

The synthesis and characterization of new metal complexes with fluoroquinolone (Levofloxacin) antibacterial agents are of great importance for understanding the drug-metal ion interaction and taking into account their potential pharmacological use. The objective of this study is the isolation and characterization of new levofloxacin vanadium (V) complexes, as well as their characterization using spectroscopic and thermal analysis techniques. The thermal behavior of these complexes was also studied. Different thermodynamic parameters were calculated using Coats–Redfern and Horowitz-Metzger methods. The antibacterial activity of the investigated complexes was tested against *E. coli, K. pneumonia, P. aeruginosa* as (Gram -ve), and *B. subtilis,S. pyogenes*, *S. aureus* as (Gram +ve).

2. Materials and methods

2.1. Chemicals

All chemicals used were of high purity grade and used without further purification. Levofloxacin was obtained from Merck Chemical Co., Ethanol, $AgNO_3$, Pyridine, Aniline, o-Tolidine, Dimethylformamide and all solvents were purchased from Fluka Chemical Co.,$VOCl_3$ (99.9%) from Aldrich Chemical Co.

2.2. Synthesis of levofloxacin vanadium complexes

An ethanolic solution (20 ml) of levofloxacin (2mmol, 0.722 g) and 2 mmol (0.080g) of NaOHwas added to an ethanolic solution of $VOCl_3$ (1 mmol, 0.190 ml) and the reaction mixture was stirred for 1h and then adding (2 ml) pyridine (1mmol, d=0.982), after that the reaction mixture was stirred for 3 days at room temperature. The solution was left for slow evaporation, after that a black $[VO(Lev)_2Py]Cl$ product was deposited. The solid obtained was filtered under vacuum, washed with ethanol and

dried. In a similar way, the brown solid complex $[VO(Lev)_2An]Cl$, and the black solid complexes $[VO(Lev)_2DMF]Cl$ and $[VO(Lev)_2o\text{-}Tol]Cl$ were prepared by using An, DMF and o-Tol instead of aniline. Unfortunately we were not able to obtained appropriate mono-crystals to perform X-ray diffraction analysis. The complexes were characterized by their elemental analysis, infrared, electronic, ^1H NMR and thermal analyses.

2.3. Instruments

Elemental C, H and N analysis was carried out on a Perkin-Elmer CHN 2400. The percentage of the V(V) was determined gravimetrically by transforming the solid products into vanadium oxide and also determined by using atomic absorption method. Spectrometer model PYE-UNICAM SP 1900 fitted with the corresponding lamp was used for this purposed. IR spectra of the prepared complexes were recorded as KBr pellets on a Perkin-Elmer FTIR type 1650 spectrophotometer in wave number region 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 in dynamic N_2 atmosphere (20mL min^{-1}) with a heating rate of 10°C/min using Shimadzu TGA-50H thermal analyzer within the temperature range from room temperature to 1200 °C. Electronic spectra were obtained using UV-3101PC Shimadzu. The solid reflection spectra were recorded. Melting points were determined on an electrothermal-9100 apparatus. Magnetic measurements were carried out on a Sherwood scientific magnetic balance using Gouy method and $Hg[Co(SCN)_4]$ as a calibrate. Molar conductivities of the prepared solutions of the ligand and their metal complexes in DMSO at 1×10^{-3} M were measured on CONSORT K410.All measurements were carried out at ambient temperature with freshly prepared solution.

2.4. Antibacterial investigation

Antibacterial activity of the ligand and its metal complexes was investigated by a previously reported modified method of Beecher and Wong **[18]**, against different bacterial species, such as *S. aureus,B. subtilis, S. pyogenes, E. coli, P. aeruginosa* and *K. pneumonia*. The tested microorganisms isolates were isolated from Egyptian soil and identified according to the standard mycological and bacteriological keys for identification of bacteria as stock cultures in the regional center for mycology and biotechnology, Al-Azhar University. The solution of 5 mg/mL of each compound in DMSO was prepared for testing against bacteria. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 104-106 CFU (colony forming unit) per ml were spread on the surface of nutrient agar (1% typetone, 0.5% Yeast extract, 0.5% NaCl, 1.0% Agar and 1000 mL of distilled water, PH 7.0) which was autoclaved under 121 ^{o}C for at least 20 min. wells were created in medium with the help of a sterile metallic bores and then cooled down to 47 ^{o}C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 µL of the tested samples (10 mg/mL) were loaded into the wells of the plates.All compounds were prepared in DMSO which was loaded as control. The plates were kept for incubation at 37 ^{o}C for 24 h and then the plates were examined for the formation of zone of inhibition. Growth inhibition was calculated with reference to the positive control, i.e., levofloxacin.

3. Results and discussion

Levofloxacin (Lev) reacted with V(V) in ethanol at room temperature to form a solid complexes with a characteristic color of the ligand. The molar ratio for all complexes synthesized is M:Lev:L=1:2:1 [L=Py, An, DMF and o-Tol] which was established from the results of the chemical analysis (Table 1). Qualitative reactions **[19]** revealed the presence of chloride ions in all complexes as counter ion. Solutions of these complexes react with silver nitrate giving a white precipitate of silver chloride indicating

the existence of chloride in the ionic form i.e., outside the coordination sphere of the metal ions. The molar conductance values of levofloxacin and metal complexes (DMSO-d_6) with standard reference using 10^{-3} M solutions at room temperature were found at 17- 86.24 S cm^2 mol^{-1} which indicated that all the prepared complexes are electrolytes in nature.

Table 1: Elemental analysis and Physico-analytical data for levofloxacin and its metal complexes.

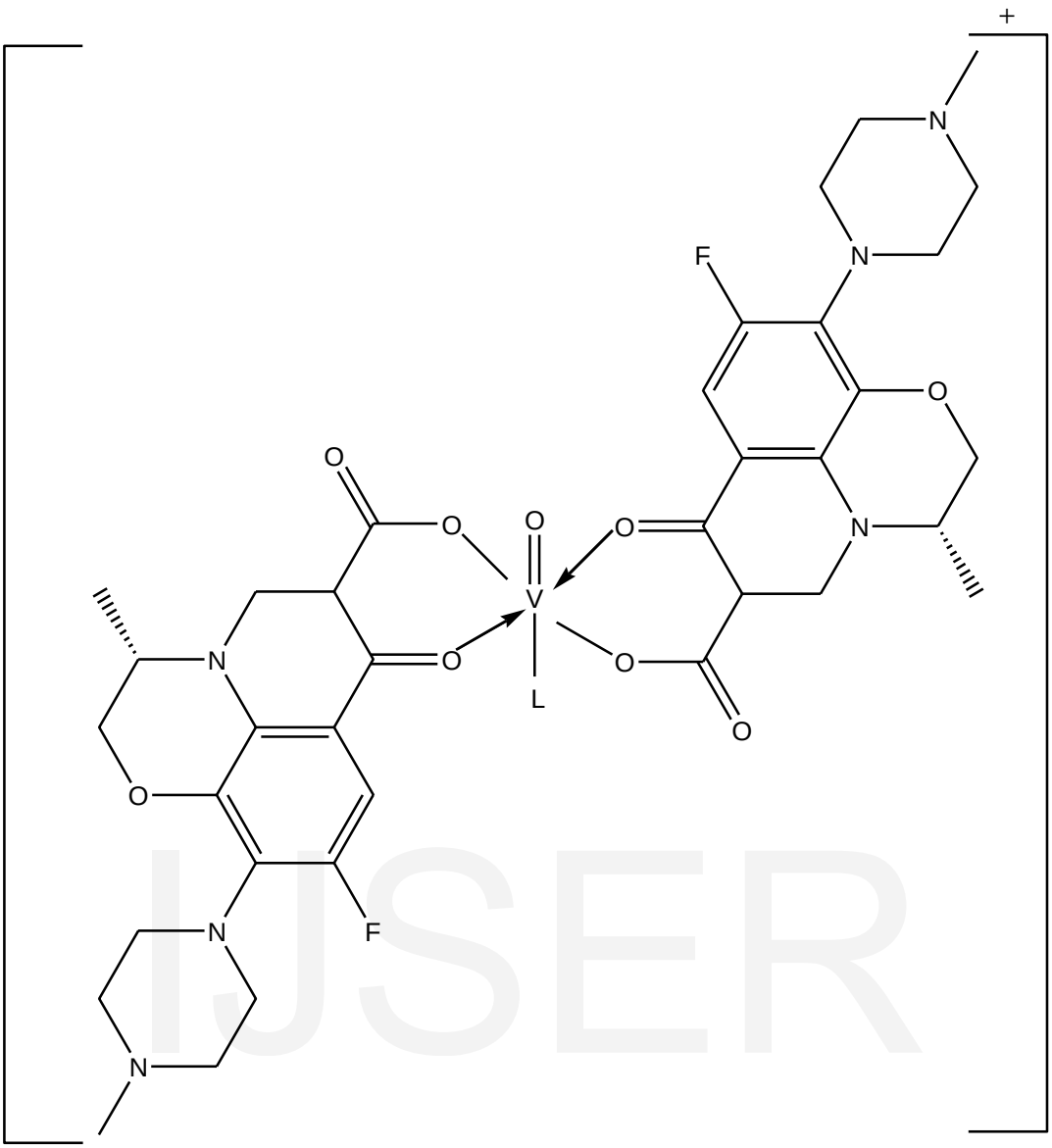
Compounds MWt. (M.F.)	Yield%	Mp/ ^{o}C	Color	Found (Calcd.) (%)					Λ (S cm^2 mol^{-1})
				C	H	N	V	Cl	
Lev 361 ($C_{18}H_{20}FN_3O_4$)	-	218	Light yellow	(59.83) 59.80	(5.54) 5.51	(11.63) 11.60	-	-	17
[VO(Lev)$_2$Py]Cl 901.44 ($VC_{41}H_{43}F_2N_7O_9Cl$)	65	284-286	Black	(54.58) 54.55	(4.77) 4.74	(10.87) 10.83	(5.65) 5.63	(3.94) 3.90	85.21
[VO(Lev)$_2$An]Cl 915.44 ($VC_{42}H_{45}F_2N_7O_9Cl$)	70	229-232	Brown	(55.05) 55.02	(4.91) 4.88	(10.70) 10.68	(5.56) 5.52	(3.88) 3.85	83.33
[VO(Lev)$_2$DMF]Cl 895.44 ($VC_{39}H_{45}F_2N_7O_{10}Cl$)	75	286-289	Black	(52.26) 52.22	(5.02) 5.00	(10.94) 10.91	(5.69) 5.65	(3.96) 3.93	84.54
[VO(Lev)$_2$o-Tol]Cl 1034.44 ($VC_{50}H_{54}F_2N_8O_9Cl$)	70	322-324	Black	(58.00) 57.96	(5.22) 5.20	(10.83) 10.80	(4.92) 4.90	(3.43) 3.40	86.24

3.1. Spectroscopic studies

3.1.1. IR absorption spectra

The IR spectra of free levofloxacin and its complexes were measured as KBr disks. The IR spectra usually provide a lot of valuable information on coordination reactions. The comparison between the levofloxacin wave numbers observed in the IR spectra of the complexes and free levofloxacin helped in defining the structure of metal complexes. The IR spectra of the complexes are very similar due to the same atom of levofloxacin involved in the bonding to the metal. The bands at 1725 and 1620 cm^{-1} correspond to $\nu(C=O)_{car}$ and $\nu(C=O)_{pyr}$, respectively. The peak at 1725 cm^{-1} corresponding to $\nu(C=O)_{car}$ of levofloxacin is disappeared in all metal complexes which indicate interaction of the carboxylato group with metal ion. Unidentate carboxylato complexes exhibit $\Delta\nu$ values >200 cm^{-1} [$\Delta\nu=\nu_{as}(COO^-)-\nu_s(COO^-)$] **[20-25]**. The observed $\Delta\nu$.is observed between 254-217 cm^{-1}, suggesting a unidentate interaction of the carboxylato group (Table 2). The band at 1620 cm^{-1} responsible for $\nu(C=O)_{pyr}$ in levofloxacin is observed between 1597 and 1524 cm^{-1} in case of complexes **[26-28]**. These data are further supported by observation of ν(M-O), ν(M-N) in the region of 629 – 444 cm^{-1}. According to the above discussion the levofloxacin is coordinated with the metal ion in all complexes as bidentate through pyridone oxygen atom and one oxygen atoms of carboxylic group.

The proposed structure formulae of the levofloxacin metal complexes according to the basis of the results discussed in this paper may be as follows (Scheme 2):



Scheme 2: The coordination mode of V(V) with Levofloxacin and L (L= Py, An, DMF and o-Tol).

Table 2: Selected infrared absorption frequencies (cm^{-1}) of ligands and its complexes.

Compound	ν(O-H); H_2O; COOH	ν(C=O); COOH	$\nu_{as}(COO^-)$	ν(C=O)	$\nu_s(COO^-)$	*ν*(V=O)	ν(M-O) and ν(M-N)
Lev	3400m	1725s	-	1620vs	-	-	-
$[VO(Lev)_2Py]Cl$	3429m	-	1643s	1524ms	1389ms	972m	598m, 578m, . 513ms, 489w, 478m
$[VO(Lev)_2An]Cl$	3400m	-	1622w	1597m	1400ms	961s	590m, 567vw 513m, 463w
$[VO(Lev)_2DMF]Cl$	3422w	-	1621w	1585m	1404ms	980vs	629w, 586m 517m, 467m
$[VO(Lev)_2o\text{-}Tol]Cl$	3422w	-	1621m	1582ms	1400ms	976vs	583w, 552w 517m, 444w

vs = very strong, s=strong, w=weak, ms= medium strong, m= mediumand υ=stretching.

3.1.2. Electronic spectra

The formation of the complexes also confirmed by UV-Vis. spectra. The UV-Vis. measurements of free levofloxacin ligand and its metal complexes have been recorded in solid state as electronic solid reflection from 200 to 800 nm. Free levofloxacin showed bands at 214 and 221nm which are assigned to π-π^*transitions these bands are shifted in complexes at 220-297 nm, also levofloxacin showed another band at 339 nm which is assigned to n-π^* transitions and is shifted in complexes at 306-381 nm. The bathochromic shift of the reflectance band and appearance of new bands for the complexes is attributed to complexation of levofloxacin.V(V). Levofloxacin complexes (Fig. 1) showed new bands at 428-571 nm which may be assigned to ligand-metal charge transfer **[27, 28]**, all these data are listed in Table 3.

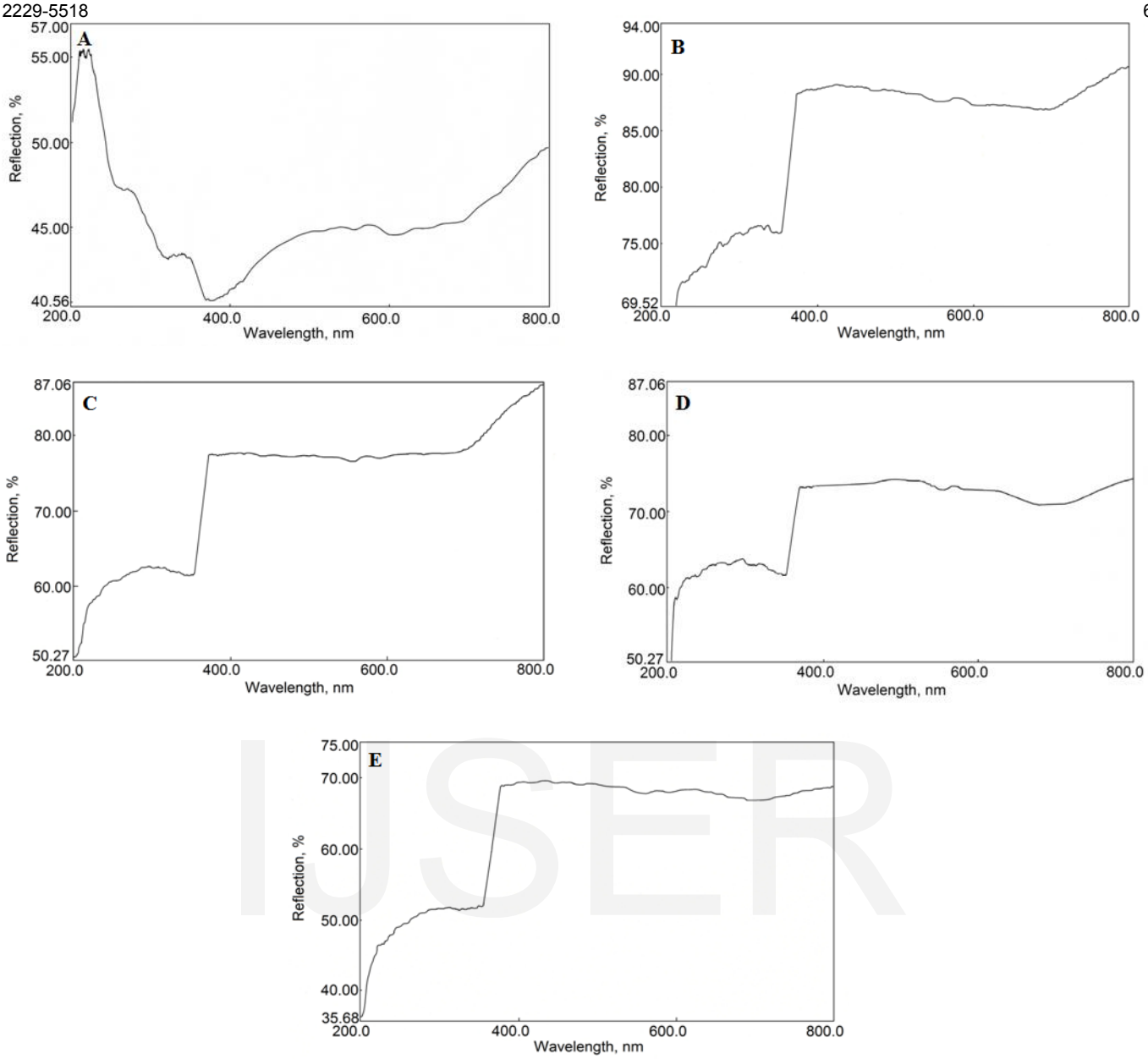


Fig. 1: Electronic reflection spectra of (A) Lev, (B) $[VO(Lev)_2Py]Cl$, (C) $[VO(Lev)_2An]Cl$,(D)$[VO(Lev)_2DMF]Cl$ and (E) $[VO(Lev)_2o\text{-}Tol]Cl$.

Table 3: UV-Vis. Spectra of (A) (Lev), (B) [VO(Lev)$_2$Py]Cl, (C) [VO(Lev)$_2$An]Cl, (D) [VO(Lev)$_2$DMF]Cl and (E) [VO(Lev)$_2$o-Tol]Cl.

Assignments (nm)	Lev	Lev complexes			
	A	B	C	D	E
π- π* transitions	214, 221	274, 297	249, 294	270, 296	220, 233
n- π* transitions	339	334, 381	307, 373	317, 374	306, 370
Ligand-metal charge transfer	-	450, 480, 490, 568	447, 476, 503, 571	495, 567	428, 448 485, 566

3.1.3. ^{1}H NMR spectra

To make sure about the proposed structureof the isolated metal complexes, the ^{1}H NMR spectra were done.The ^{1}H NMR spectra data of levofloxacin and its complexes with V(V) (Fig. 2) in DMSO-d_6 and their peaks assignments are listed in Table 4. The resonance of carboxylic proton (COOH) is not detected in the spectra of the our complexes while it was appeared in levofloxacin spectrum (at 11.0ppm) this indicated the binding of carboxylate group with vanadium ion. However, resonance of aryl protons appeared down field near 8.4–8.5 and 7.65–7.9 ppm in the spectra of all complexes. The shift is due to the complexation and difference in the configuration of complexes than ligand **[29]**. The change of the signals for the aliphatic and piperazine protons are practically small since they lie far from the binding site of the ligand **[30]**.

Therefore, the data indicated that levofloxacin acts as a ligand through the carboxylic group. Our studies suggest that the piperazine nitrogen does not interact with metal probably due to the steric crowding of the methyl group **[31]**.

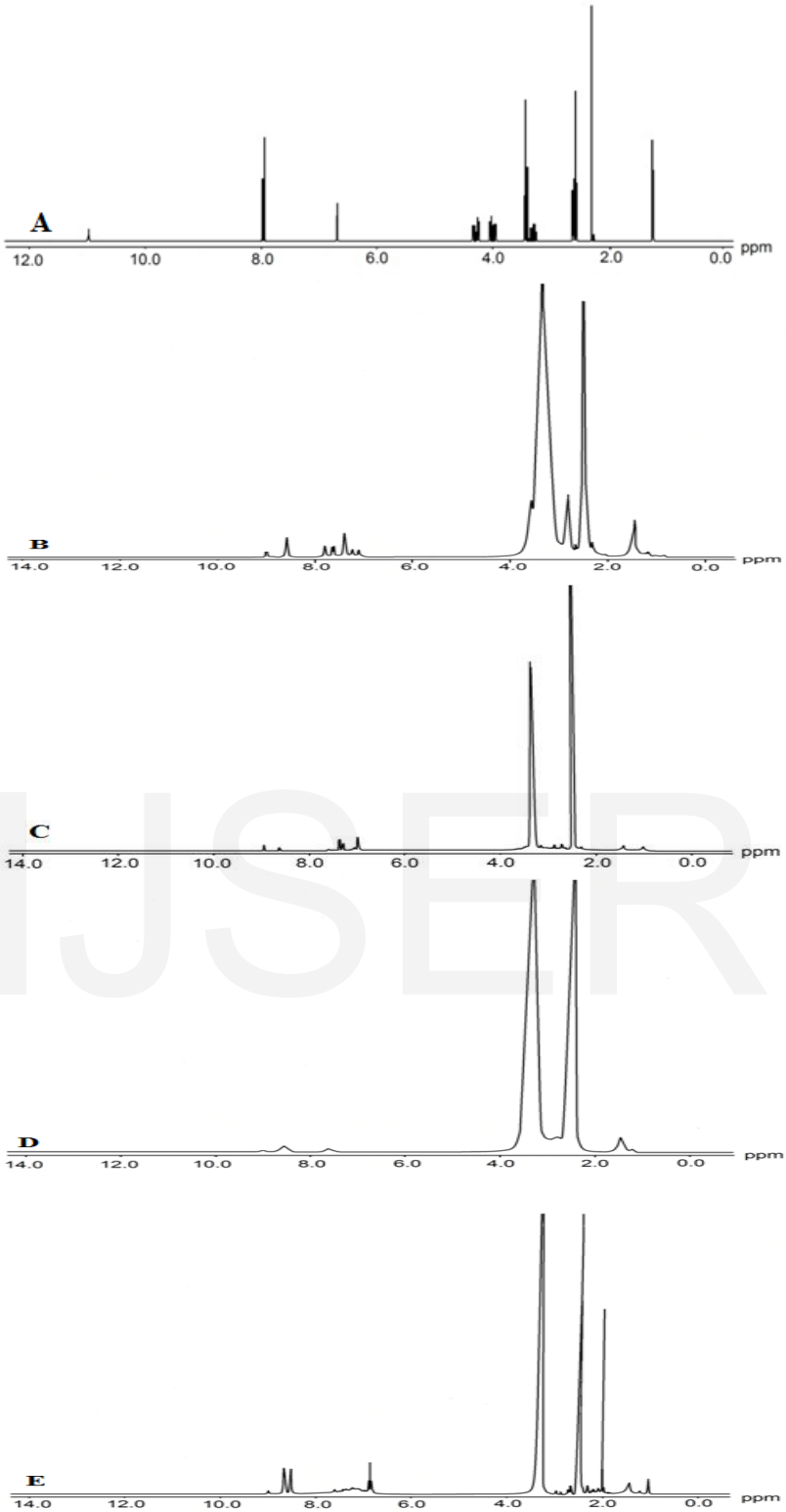


Fig. 2: ^{1}H NMR spectra of (A) Lev, (B) [VO(Lev)$_2$Py]Cl, (C) [VO(Lev)$_2$An]Cl, (D)[VO(Lev)$_2$DMF]Cl and (E) [VO(Lev)$_2$o-Tol]Cl.

Table 4: ^{1}H NMR values (ppm) and tentative assignments for (A) Lev, (B) [VO (Lev)$_2$Py]Cl, (C) [VO(Lev)$_2$An]Cl, (D) [VO(Lev)$_2$DMF]Cl and (E) [VO(Lev)$_2$o-Tol]Cl.

A	B	C	D	E	Assignments
1.4	1.18, 1.45	1.02-1.46	1.24, 1.45	1.02-1.98	δH, -CH_3
2.19-2.53	2.32-2.81	2.32-2.877	2.49, 2.81	2.02-2.97	δH, -CH_2 aliphatic
3.23-3.37	3.37, 3.56	3.15-3.48	3.36	3.34, 3.44	δ H, -N-CH_2
7.52-8.93	7.10-9.2	7.043-8.99	7.62-8.99	7.23-8.99	δH, -CH_2 aromatic
11.0	-	-	-	-	δH, -COOH

3.1.4. Thermal studies

Thermogravimetric (TGA) and differential thermogravimetric (DTG) analyses of the ligand and their solid complexes were carried out to get an information about the thermal stability of these new complexes and to suggest a general scheme for thermal decomposition. The heating rates were suitably controlled at 10 ^{o}C min^{-1} under N_2 atmosphere with rate flow 20 ml min^{-1} and the weight loss was measured from the ambient temperature up to ~ 1000 ^{o}C. The thermal analyses curves are shown in Fig. 3 and the data are tabulated in Table 5.

The thermal degradation for the ligand (Lev) started at 42 ^{o}C and finished at 946^{o}C with two stages. The first stage of decomposition occurs at maximum temperature of 75^{o}C and accompanied by a weight loss of 7.20% which may be attributed to the loss of C_2H_2 molecule.The second stage of decomposition occurs at two maxima 341 and 531^{o}C and the weight loss found at this stage equals to 92.80% corresponding to loss $7C_2H_2$+HF+$2CO_2$+N_2+NH_3.

The thermal decomposition of [VO(Lev)$_2$Py]Cl complex started at 32 ^{o}C and finished at 980 ^{o}C with two decomposition steps, the first stage at a temperature

maximum of 68 °C and accompained by weight loss of 8.71% which may be attributed to the loss of Py molecule that is in good agreement with the calculated values of 8.76%.The second stage with two maxima 324 and 485 °C accompained by weight loss of 67.82% which corresponds to loss of levofloxacin molecules and giveing the final product as $0.5V_2O_5$+10C.

The thermal decompostion of $[VO(Lev)_2An]Cl$ complex proceeds with two main degradation steps. The first stage of decomposition occurs at maximum temperature value 63 °C and is accompained by weight loss of 10.11%, corresponding to the loss of An molecule.The next decomposition step occurs at two maxima 449 and 792 °C, with a weight loss of 69.35% giving$0.5V_2O_5$+8Cas a final product.

For $[VO(Lev)_2DMF]Cl$ complex, the thermal decomposition exhibits two main degradation steps. The first stage of decomposition starting from room temperature up to 199 °C at temperature maximum of 95 °C is accompanied by a weight loss of 8.12% in agreement with the theoretical values 8.15% for the loss of the DMF molecule. The second step occurs at temperature maximum of 381 °C with a weight loss of 73.64%, giving $0.5V_2O_5$+6C as a final product.

The thermal degradation for $[VO(Lev)_2$o-Tol$]Cl$ complex, exhibits approximately two main degradation steps. The first stage of decomposition occurs from 27 to 236 °C with two maxima of 68 and183 °C and accompanied by a weight loss of 20.47% in agreement with the theoretical values 20.49% for the loss of o-Tol molecule. The second step of decompostion occurs at two maxima 579 and 714 °C with a weight loss of 62.58% and the final product is $0.5V_2O_5$+7C.

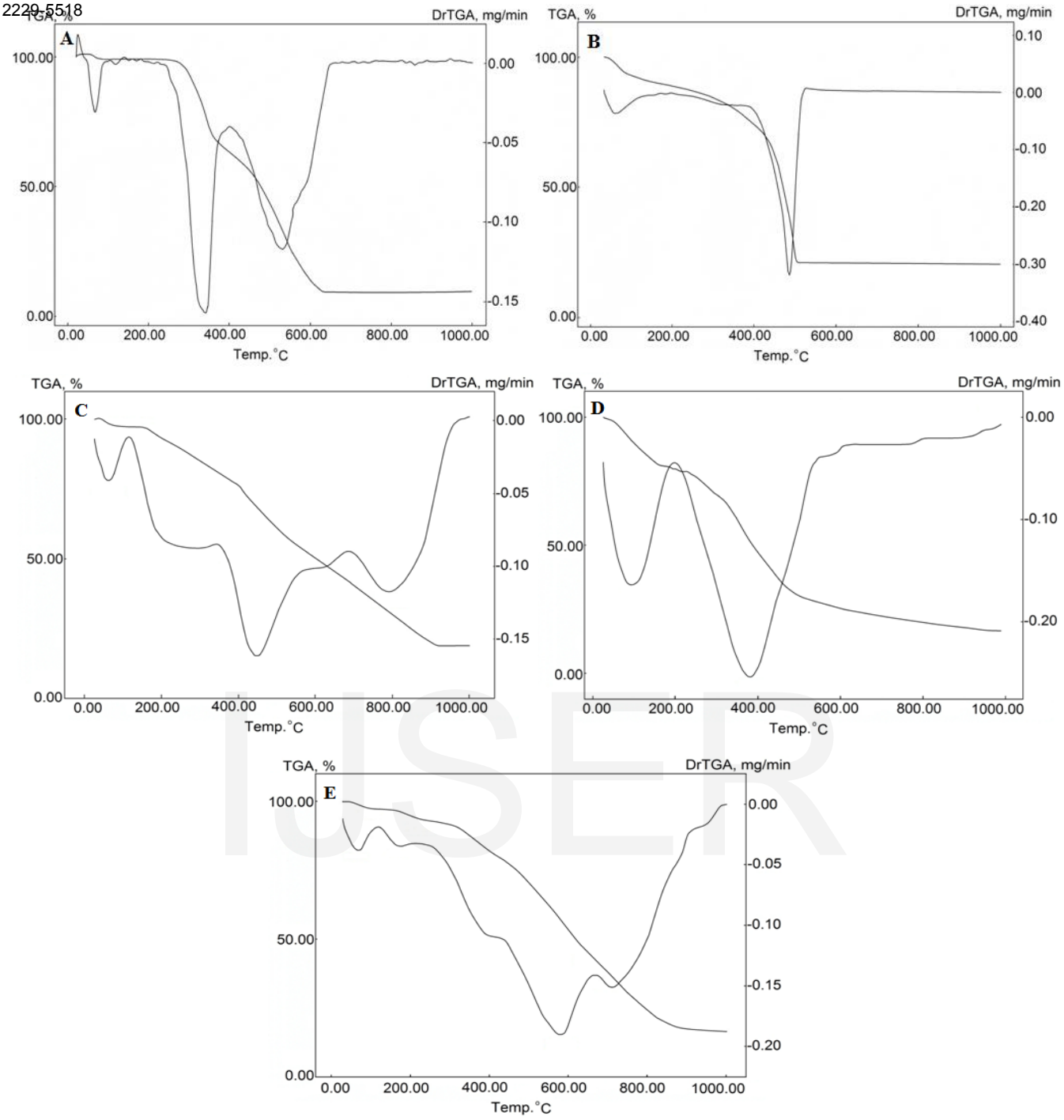


Fig. 3: TGA and DTG diagrams of (A) Lev, (B) $[VO(Lev)_2Py]Cl$, (C) $[VO(Lev)_2An]Cl$, (D)$[VO(Lev)_2DMF]Cl$ and (E) $[VO(Lev)_2o\text{-}Tol]Cl$.

Table 5: The maximum temperature T_{max}(°C) and weight loss values of the decomposition stages for Lev and V(V) complexes.

Compounds	Decomposition Steps	T_{max}(°C)	Weight loss (%)		Lost species
			Calc.	Found	
Lev ($C_{18}H_{20}N_3O_4F$)	First step Second step Total loss Residue	75 341, 531	7.20 92.80 100.00 0.00	7.20 92.80 100.00 0.00	C_2H_2 $7C_2H_2+2CO2+HF+N_2+NH_3$
[VO(Lev)$_2$Py]Cl ($VC_{41}H_{43}F_2N_7O_9Cl$)	First step Second step Total loss Residue	68 324, 485	8.76 67.84 76.60 23.40	8.71 67.82 76.53 23.47	$2.5C_2H_2+ 0.5N_2$ $4C_2H_4+9C_2H_2+HCl+2HF+0.5H_2O+6NO$ $0.5V_2O_5+10C$
[VO(Lev)$_2$An]Cl ($VC_{42}H_{45}F_2N_7O_9Cl$)	First step Second step Total loss Residue	63 449, 792	10.16 69.42 79.58 20.42	10.11 69.39 79.50 20.50	$3C_2H_2+0.5N_2+0.5H_2$ $11C_2H_2+3C_2H_4+2HF+HCl+0.5H_2O+6NO$ $0.5V_2O_5+8C$
[VO(Lev)$_2$DMF]Cl ($VC_{39}H_{45}F_2N_7O_{10}Cl$)	First step Second step Total loss Residue	95 381	8.15 73.66 81.81 18.19	8.12 73.64 81.76 18.24	$C_2H_6+CO+0.5N_2+0.5H_2$ $13C_2H_2+2C_2H_4+2HF+HCl+0.5H_2O+6NO$ $0.5V_2O_5+6C$
[VO(Lev)$_2$o-Tol]Cl ($VC_{50}H_{54}F_2N_8O_9Cl$)	First step Second step Total loss Residue	68, 183 579, 714	20.49 62.60 83.09 16.91	20.47 62.58 83.05 16.95	$7C_2H_2+H_2+N_2$ $14C_2H_2+CO_2+2HF+HCl+2NH_3+0.5H_2O+4NO$ $0.5V_2O_5+7C$

3.1.5. Kinetic and thermodynamic parameters

The kinetic and thermodynamic parameters were determined by non isothermal methods. The non-isothermal kinetic analysis for the thermal decomposition of all ligands and complexes in this work was carried out by the application of the Coats-Redfern **[32]** and Horowitz-Metzger methods **[33]**.

Coats–Redfern equations

$$\ln X=\ln\left[\frac{-\ln(1-\alpha)^{1-n}}{T^2(1-n)}\right]=\frac{-E^*}{RT}+\ln\left[\frac{AR}{\varphi E^*}\right] \text{ for } n\neq 1 \quad (1)$$

where (n=0, 0.33, 0.5 and 0.66)

$$\ln X=\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]=\frac{-E^*}{RT}+\ln\left[\frac{AR}{\varphi E^*}\right] \text{ for } n=1 \quad (2)$$

Horowitz-Metzger equations

$$\ln X=\ln\left[\frac{-\ln(1-\alpha)^{1-n}}{T^2(1-n)}\right]=\frac{-E^*}{RT}+\ln\left[\frac{AR}{\varphi E^*}\right] \text{ for } n\neq 1 \quad (3)$$

where (n=0, 0.33, 0.5 and 0.66)

$$\ln X=\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right]=\frac{-E^*}{RT}+\ln\left[\frac{AR}{\varphi E^*}\right] \text{ for } n=1 \quad (4)$$

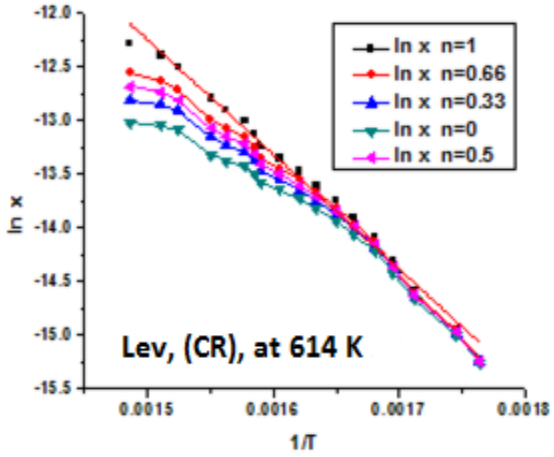
The thermodynamic parameters: entropy change (ΔS*), enthalpy change (ΔH*) and free energy of activation change (ΔG*) were calculated using the following equations:

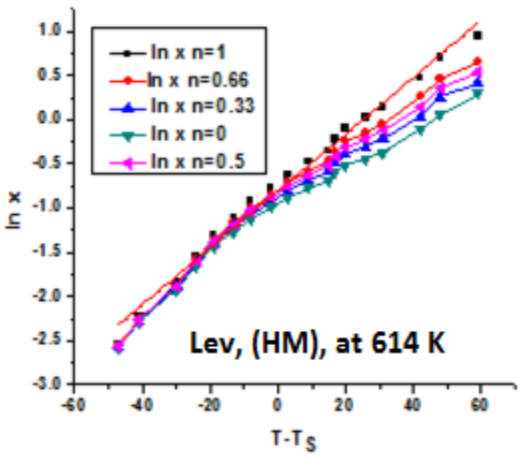
$$\Delta S^*=R\ln(Ah/k_BT_s) \quad (5)$$

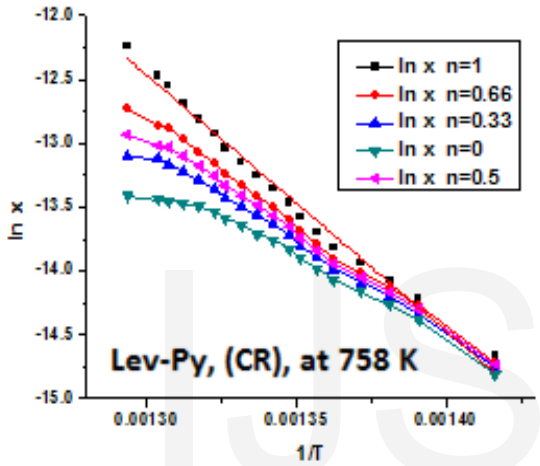
$$\Delta H^*=E^*-RT \quad (6)$$

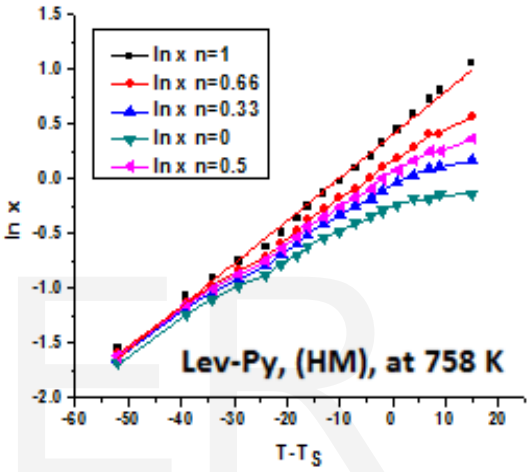
$$\Delta G^*=\Delta H^*-T\Delta S^* \quad (7)$$

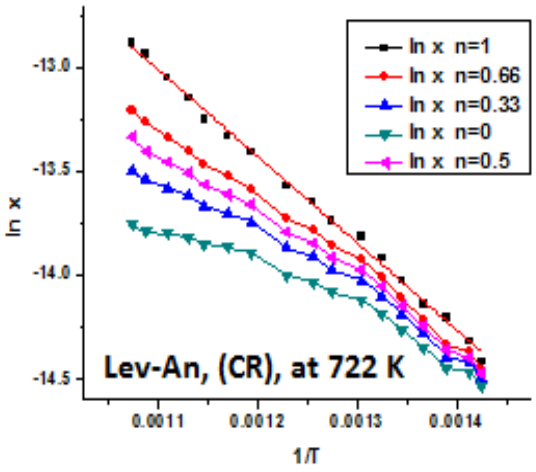
The TGA (TG and DTG) curves recorded for Lev and its complexes are given in Fig. 4. The data are summarized in Table 6. The activation energies of decomposition were found to be in the range 34.71-166.57 KJ mol^{-1}.The entropy of activation is found to have negative values in all the complexes which indicate that the decomposition reactions proceed with a lower rate than the normal ones.

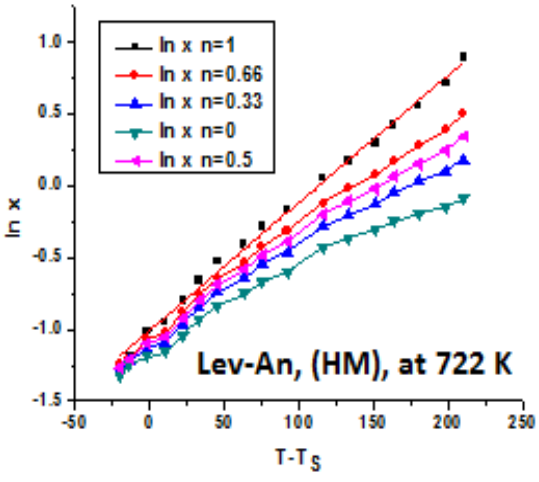

ln x n=1
ln x n=0.66
ln x n=0.33
ln x n=0
ln x n=0.5
Lev, (CR), at 614 K
ln x
1/T


ln x n=1
ln x n=0.66
ln x n=0.33
ln x n=0
ln x n=0.5
Lev, (HM), at 614 K
ln x
T-T S


ln x n=1
ln x n=0.66
ln x n=0.33
ln x n=0
ln x n=0.5
Lev-Py, (CR), at 758 K
ln x
1/T


ln x n=1
ln x n=0.66
ln x n=0.33
ln x n=0
ln x n=0.5
Lev-Py, (HM), at 758 K
ln x
T-T S


ln x n=1
ln x n=0.66
ln x n=0.33
ln x n=0
ln x n=0.5
Lev-An, (CR), at 722 K
ln x
1/T


ln x n=1
ln x n=0.66
ln x n=0.33
ln x n=0
ln x n=0.5
Lev-An, (HM), at 722 K
ln x
T-T S

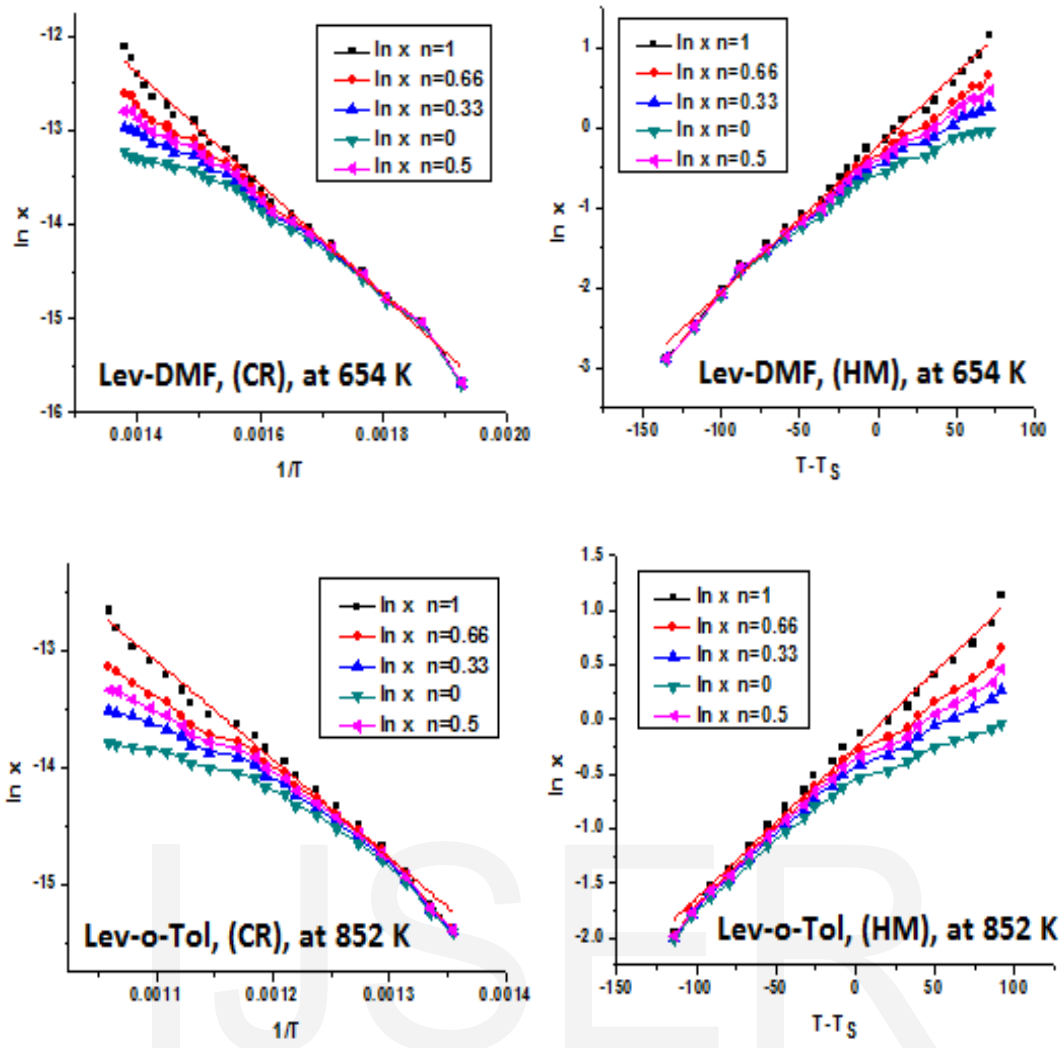


Fig. 4: The diagrams of kinetic parameters of levofloxacin and its metal complexes using Coats-Redfern (CR) and Horowitz-Metzger (HM) equations.

Table 6: Thermal behavior and Kinetic parameters determined using Coats–Redfern (CR) and Horowitz–Metzger (HM) operated for Lev and its complexes.

Compounds	Decomposition Range (K)	T_s (K)	method	parameter					R^a	SD^b
				E^* (KJ/ mol)	A (s^{-1})	ΔS^* (KJ/mol.K)	ΔH^* (KJ/mol)	ΔG^* (KJ/mol)		
Lev	482-680	614	CR	88.88	7.88×10^4	-0.1572	83.77	180.27	0.995	0.086
($C_{18}H_{20}N_3O_4F$)			HM	100.61	1.94×10^6	-0.1305	95.51	175.66	0.994	0.103
[VO(Lev)$_2$Py]Cl	522-780	758	CR	166.57	2.62×10^9	-0.0724	160.26	215.11	0.994	0.069
($VC_{41}H_{43}F_2N_7O_9Cl$)			HM	187.69	5.63×10^{10}	-0.0469	181.38	216.91	0.997	0.052
[VO(Lev)$_2$An]Cl	633-960	722	CR	34.71	3.16×10^6	-0.1278	28.71	121.01	0.998	0.026
($VC_{42}H_{45}F_2N_7O_9Cl$)			HM	82.78	3.10×10^3	-0.1854	76.78	210.64	0.997	0.047
[VO(Lev)$_2$DMF]Cl	505-733	654	CR	49.27	5.95×10^4	-0.1600	43.83	148.50	0.996	0.077
($VC_{39}H_{45}F_2N_7O_{10}Cl$)			HM	64.72	4.48×10^2	-0.2007	59.28	190.53	0.997	0.079
[VO(Lev)$_2$o-Tol]Cl_3	715-974	852	CR	69.93	6.55×10^4	-0.1614	62.84	200.39	0.995	0.071
($VC_{50}H_{54}F_2N_8O_9Cl$)			HM	83.47	3.02×10^2	-0.2062	76.38	252.03	0.996	0.074

a=correlation coefficients of the Arrhenius plots and b=standard deviation

3.1.6. Antibacterial activities

The susceptibility of certain strains of bacterium towards levofloxacin and its complexes was judged by measuring size of the inhibition zone diameter, because these microorganisms can get resistance to antibiotics and their metal complexes through biochemical and morphological modifications **[29]**. Antibacterial activies of levofloxacin and its complexes have been carried out with three Gram-positive bacteria such as *B. subtilis*, *S. pyogenes* and *S. aureus* and three Gram-negative species *E. coli*, *K.pneumoniae* and *P. aeruginosa*. The tests solutions were prepared in DMSO-d_6 and the results are presented in Table 7 and Fig. 5.

A comparative study of ligand and their metal complexes showed that the $[VO(Lev)_2o\text{-}Tol]Cl$ complex exhibit higher antibacterial activity against three types of Gram-positive bacteria and two types of Gram-negative bacteria and no activity observed for *P. aeruginosa*, while the other complexes showed an excellent activity against all microorganisms.

Chelation considerably reduced the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the chelate ring **[30]**. Such chelation increased the lipophilic character of the central metal ion, which subsequently favors the permeation through the lipid layer of cell membrane. This increased lipophilicity enhances the penetration of the complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms **[34]**.

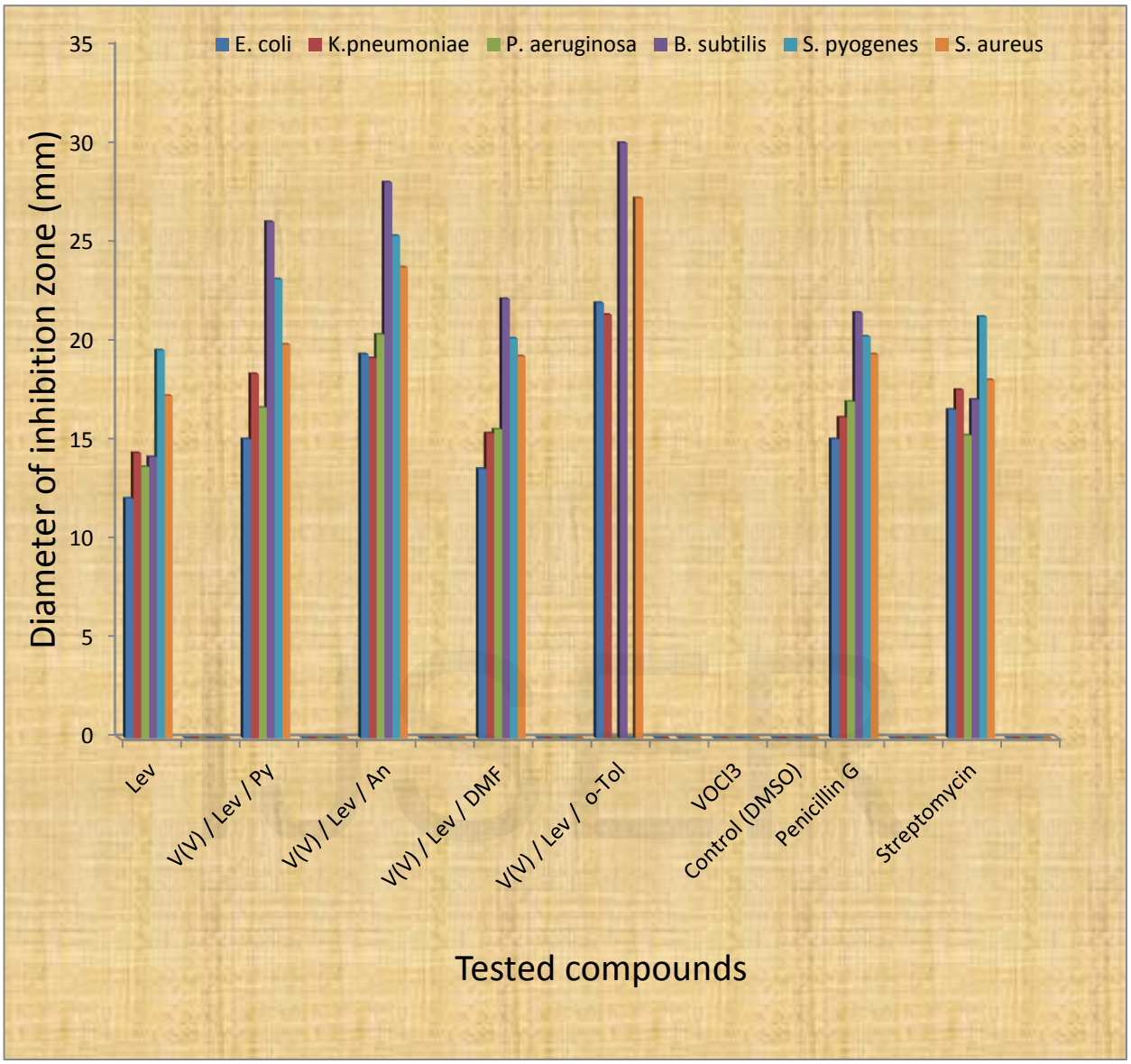


Fig. 5: Statistical representation for biological activity of levofloxacin and its complexes.

Table 7: The inhibitation diameter zone values (mm) for Lev and its complexes.

Compounds		Microbial Bacteria species					
		E. coli	*K.pneumoniae*	*P. aeruginosa*	*B. subtilis*	*S. pyogenes*	*S. aureus*
Lev		12.1 ± 0.8	14.4 ± 0.2	13.7 ± 0.5	14.2 ±0.4	19.6 ±0.3	17.3 ±0.7
V(V) / Lev / Py		15.1^{+1} ±0.04	18.4^{+1} ±0.03	16.7^{+1} ±0.03	26.1^{+2} ±0.01	23.2^{+1} ±0.05	19.9^{+1} ±0.05
V(V) / Lev / An		19.4^{+1} ±0.05	19.2^{+1} ±0.01	20.4^{+2} ±0.07	28.1^{+2} ±0.01	25.4^{+1} ±0.03	23.8^{+1} ±0.1
V(V) / Lev/ DMF		13.6^{NS} ±0.03	15.4^{NS} ±0.08	15.6^{+1} ±0.1	22.2^{+1} ±0.08	20.2^{+1} ±0.04	19.3^{+1} ±0.09
V(V) / Lev/ o-Tol		22.0^{+2} ±0.3	21.4^{+2} ±0.09	NA	30.1^{+3} ±0.09	$29,4^{+3}$ ±0.09	27.3^{+2} ±0.2
$VOCl_3$		0	0	0	0	0	0
Control (DMSO)		0	0	0	0	0	0
Standard	Penicillin G	15.1 ±0.07	16.2 ±0.09	17.0 ±0.05	21.5 ±0.06	20.3 ±0.2	19.4 ±0.08
	Streptomycin	16.6 ±0.04	17.6 ±0.09	15.3 ±0.08	17.1 ±0.04	21.3 ±0.2	18.1 ±0.08

NA: No activity, data are expressed in the form of mean ± SD.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).

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