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SYNTHESIS, SPECTROSCOPIC, THERMAL AND BIOLOGICAL ACTIVITY INVESTIGATION OF NEW Y(III) AND Pd(II) NORFLOXACIN COMPLEXES

Sadeek A. Sadeek[1♥], Walaa H. El-Shwiniy[1], Wael A. Zordok[1] and Akram M. El-Didamony[1]

[1]*Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt*

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Abstract

Two new complexes were prepared from the interaction of Y(III) and Pd(II) with norfloxacin (NOR) in ethanol and diethyl ether at room temperature and separated as solids with characteristic colors. The infrared spectra were obtained and full assignment of all the observed vibrations is proposed which indicate that the norfloxacin react as bidentate ligand through the carbonyl oxygen atom and one of oxygen atoms of the carboxylate group forming six membered rings with the metal ions. The complexes were characterized by using ^{1}HNMR and UV-vis spectroscopies, as well as thermal analysis. The activation energies, E^{*}, entropies, ΔS^{*}, enthalpies, ΔH^{*} and Gibbs free energies, ΔG^{*}, of the thermal decomposition reactions have been derived from thermogravimetric (TGA) and differential thermogravimetric (DrTGA) curves, using Coats-Redfern and Horowitz-Metzger methods. The proposed structure of the two complexes were detected by using the Density Functional Theory (DFT) at the B3LYP/CEP-31G level of theory. The ligand as well as their metal complexes were also evaluated for their antibacterial activity against several bacterial species, such as *Staphylococcus aureus (S. aureus*), *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* and antifungal screening was studied against two species: *penicillium (P. rotatum)* and

♥Corresponding author. E-mail: sadeek59@yahoo.com

trichoderma (T. sp.)). This study showed that the metal complexes are more effective as antibacterial agents as compared to uncomplexed ligand and no antifungal activity observed for ligand and their complexes. The present study was carried out in search of a target antibiotic instead of a broad spectrum one.
Keywords: norfloxacin complexes; infrared spectra; thermal analysis; antibacterial activity.

Resumen
Dos nuevos complejos fueron preparados a partir de la interacción de Y(III) y Pd(II) con norfloxacina (NOR) en etanol y dietiléter a temperatura ambiente y separados como sólidos con colores característicos. Se obtuvieron los espectros infrarrojos y proponiendo la asignación completa de todas las vibraciones observadas lo que indica que la norfloxacina reacciona como ligando bidentado a través del átomo de oxígeno carbonílico y uno de los átomos de oxígeno del grupo carboxilato formando anillos de seis átomos con los iones metálicos. Los complejos se caracterizaron mediante el uso de ^{1}HNMR y espectroscopía UV-visible, así como análisis térmico. Las energías de activación, E*, entropías, ΔS*, entalpías, ΔH* y energías de libre de Gibbs, ΔG*, de las reacciones de descomposición térmica han sido derivadas de las curvas termogravimétricas (TGA) y curvas de termogravimetría diferencial (DrTGA), utilizando los métodos de Coats-Redfern y Horowitz.-Metzger. La estructura propuesta de los dos complejos fue corroborada mediante el uso de la teoría funcional de densidad (DFT) en el nivel teórico B3LYP/CEP-31G. El ligando, así como sus complejos metálicos también fueron evaluados por su actividad antibacteriana frente a varias especies bacterianas, tales como *Staphylococcus aureus* (S. *aureus*), *Escherichia coli* (*E. coli*) y *Pseudomonas aeruginosa* (*P. aeruginosa*) mientras que la protección antimicótica fue estudiada frente a dos especies: *penicillium* (*P. rotatum*) y *trichoderma* (*T. sp*.). Este estudio mostró que los complejos metálicos son más eficaces como agentes antibacteriales, en comparación con el ligando no complejado además de no observar ninguna actividad antimicótica para el ligando y sus complejos. El presente estudio se llevó a cabo con el objetivo de la búsqueda de un antibiótico específico en lugar de uno de amplio espectro.
Palabras clave: complejos de norfloxacina; espectros infrarrojo; análisis térmico; actividad antibacterial.

Introduction

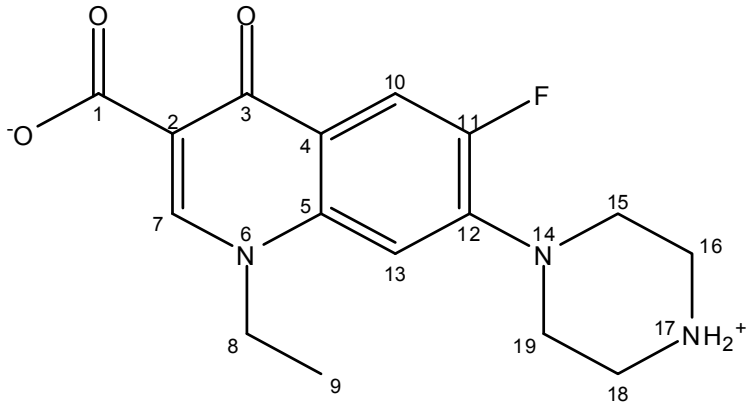
Norfloxacin (NOR) is member of the second generation quinolone antibiotics family, which are a clinically important antibacterial agent derived from Nalidixic acid, it is a specific inhibitor of DNA gryase, a bacterial type II topoisomerase and topoisomerase IV, which unwind the supercoiled DNA prior to replication and transcription [1]. All of the quinolone antibiotics share 3-oxo-1-carboxylic acid groups (Scheme I) which are essential for their bacterial activity.

The synthesis and chemistry of quinolone complexes of metal ions have been investigated extensively [2-7]. The crystal structures of quinolone complexes indicate that quinolone antibiotics can participate in the formation of complexes in a number of ways [7-13]. The neutral quinolones in the zwitterionic state (Scheme I) are potentially capable to coordinate with metal ions as a bidentate ligand forming simple complexes via one of the oxygen atoms of the carboxylate group at position one and through the ring carbonyl group at position 3 [7,8,11-13]. The quinolones can also forming polynuclear complexes when react with metal ions as bridging ligands [9]. In acidic medium the isolated complexes usually contain single or doubly protonated quinolones that are incapable of bonding to metal ions and, in such cases, only electrostatic interaction are observed between the metal ions and drug [8-10].

Infrared spectroscopy has been widely used as a powerful means for determination the site and kind of donation to metal ions. Density Functional Theory (DFT) at the B3LYP/CEP-31G level of theory was used to compute the cation type influence on theoretical parameters and detect the exact structure of the compounds. Such computational characterization reduces time consuming experiments for biomedical and pharmaceutical studies of the drugs and its complexes. Profiles of

the optimal set and geometry of these complexes were simulated by applying the GAUSSIAN 98W package of programs [14] at B3LYP/CEP-31G [15] level of theory.

To continue our investigation in the field of quinolone complexes [3,4], we report in the present article, the synthesis and characterization of new metal complexes formed from the interaction of NOR with Yttrium(III) and Palladium(II) in the solvent and study the effect of oxidation state of the two ions on the biological activity of norfloxacin drug. The isolated solid complexes are characterized using spectroscopic and thermal analysis techniques. The thermal behavior of our complexes was also studied and the antibacterial activity was also tested against several bacterial species, such as *Staphylococcus aureus (S. aureus*), *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* and antifungal screening was studied against two species: *penicillium (P. rotatum)* and *trichoderma (T. sp.)*.



Scheme I: Structure of norfloxacin (NOR) and its zwitterionic structure.

Experimental

Chemicals

All chemicals used for the preparation of the complexes were of analytical reagent grade, commercially available, used without further purification. Norfloxacin was obtained from Merck Chemical Co.while $PdCl_2$ and YCl_3 were obtained from Aldrich Chemical Co.

Synthesis

The pale white solid complex $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ was prepared by adding 0.5mmol (0.0977g) of Yttrium chloride (YCl_3) in 10ml bidistilled water drop wisely to a stirred suspended solution of 1mmol (0.3193g) of NOR in 50ml ethanol. The reaction mixture was stirred for 15 h at 35 ^oC. The pale white precipitate was filtered off and dried in vacuum over $CaCl_2$. The white brown solid complex of $[Pd(NOR)_2]Cl_2.3H_2O$ was prepared in a similar manner described above by using diethyl ether as a solvent instead of ethanol and using $PdCl_2$ in 1:2 molar ratio. In order to verify that the chloride is ionic and not coordinated, the complexes solutions were tested with an aqueous solution of $AgNO_3$ (a precipitate was formed) and the percentage of chloride ions was estimated volumetrically according to the known method [16]. The two complexes were characterized by their elemental analysis, infrared, electronic, ^{1}HNMR, thermal analysis as well as Density Functional Theory (DFT) at the B3LYP/CEP-31G level of theory.

Instruments

Elemental C, H, N and halogen 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 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 were recorded on FTIR 460 PLUS (KBr discs) in the range from 4000-400 cm^{-1}, ^{1}HNMR 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. Electronic spectra were obtained using UV-3101PC Shimadzu with a 1cm quartz cell. Molar conductivities in DMSO at 1.0×10^{-3} M were measured on CONSORT K410.

Antimicrobial investigation

Antibacterial activity of the complexes/ligand was investigated by a previously reported modified method of Beecher and Wong [17], against different bacterial species, such as *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* and antifungal screening was studied against two species (*penicillium (P. rotatum)* and *trichoderma (T. sp.)*).The microorganisms were purchased from the laboratory of (Microbiology) in the 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 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 and then cooled to 47^oC and seeded with tested microorganisms. After solidification 5mm diameter holes were punched by a sterile cork-borer. The investigated compounds, i.e., ligand and their complexes, were introduced in Petri-dishes (only 0.1ml) after dissolving in DMSO at 1.0×10^{-3} M. The plates were then incubated for (20h at 37 ^oC for bacteria and for 7 days at 30 ^oC for fungi). At the end of incubation period the inhibition zone around each hole was measured.

Results and discussion

Y(III) and Pd(II) norfloxacinate were prepared and isolated as solids of a color characteristic of the metal ions. The molar ratio for all complexes is M: NOR=1:2 which was established from the results of the chemical analysis, Table 1, and the number of bound water molecules in these compounds being different. Qualitative reactions revealed the presence of chloride as counter ions in the two complexes.

The elemental analysis results are summarized in Table 1. These results, as well as the obtained thermal analysis are in good agreement with the proposed formula. The melting points of the complexes are much more than that of the free ligand, revealing that the complexes are much more stable than ligand. The molar conductance values of the Y(III) and Pd(II) complexes were found at 244.4 and 116.48 S cm^2 mol^{-1}, which indicates that the complexes are electrolytes [18]. The low conductivity values are in agreement with the low solubility of NOR complexes in water, ethanol, chloroform, acetone and most organic solvents. On the other hand, they are soluble in DMSO, DMF and concentrated acids.

IR data and bonding

The infrared spectra of the two complexes are compared with those of the free ligand, Table 2 and Figure 1, in order to determine the site of coordination that may be involved in chelation. The presence of the broad bands in the 3434-3366 cm^{-1} zone confirms the presence of water molecules in the prepared complexes, Table 2, and the presence of a group of weak and medium intensity

bands around 2819 and 2840 cm^{-1} which assigned to vibration of the quaternized nitrogen of the piperazine group, indicates the zwitterionic form of NOR is involved in the coordination to the metal ions investigated [19]. The two bands observed at 1729 and 1619 cm^{-1} in the spectrum of the free NOR have been assigned before to the stretching vibration of carboxylic ν(COOH) and the carbonyl group ν(C=O) , respectively, [3-5,10,20-23].

Table 1. Physico-analytical data of metal norfloxacin complexes.

Complexes (M.Wt.)	**Yield%**	**mp/ °C**	**Color**	**Content (calculated) found**					**Λ (S cm^2 mol^{-1})**
				% C	% H	% N	%Cl	%M	
$[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ (1049.4)	67.45	>360	Pale white	(36.59) 36.66	(5.72) 5.46	(8.00) 8.23	(10.15) 10.14	(8.47) 8.40	244.4
$[Pd(NOR)_2]Cl_2.3H_2O$ (869.4)	71.73	250	White brown	(44.17) 44.14	(4.83) 4.9	(9.66) 9.74	(8.17) 8.15	(12.24) 12.00	116.48

The disappears of the band at 1729 cm^{-1} and the shift of the characteristic peak of the carbonyl group ν(C=O) to a lower value, Table 2, from 1619 cm^{-1} to 1569 cm^{-1} for Y(III) and 1583 cm^{-1} for Pd(II) indicates coordination of NOR through one of the oxygen atom of the carboxylate group and the oxygen atom of the carbonyl group. The asymmetric stretching carboxylate bands appear at 1630 and 1621cm^{-1} for Y(III) and Pd(II) complexes. The spectra of our two complexes also show two medium strong bands at 1403 and 1383 cm^{-1}. These bands are absent in the spectrum of NOR and most likely due to the symmetric vibration of the ligated COO^- group. Unidentate carboxylate complexes exhibit Δν values<200 cm^{-1} [$\Delta\nu=\nu_{as}(COO^-)-\nu_s(COO^-)$] [22,23]. The observed Δν for Y(III) and Pd(II) NOR complexes are around 233 cm^{-1} , Table 2, suggesting a unidentate interaction of the carboxylate group.

New bands are found in the spectra of the complexes at 565, 514 cm^{-1} for Y(III) and at 557, 520 cm^{-1} for Pd(II), which are assigned to ν(M-O) stretching vibrations of coordinated carboxylate oxygen atom and carbonyl oxygen atom. According to the above discussion the NOR is coordinated to metal ions as a bidentate ligand through the oxygen atom of the carboxylate group and the oxygen atom of the carbonyl group.

UV-Vis. spectra

The electronic solid reflection spectra of the norfloxacin along with those of the formed solid complexes are shown in Figure 2. The reflection spectrum of free norfloxacin appears at 252, 316 and 355 nm, while the reflection spectra for Y(III) and Pd(II) norfloxacins show two bands for each complex which found at 320, 432 nm for Y(III) and at 302, 334 nm for Pd(II). The shift of the reflectance λ_{max} to higher values (*bathochromic shift*) for Y(III) and to lower values (*hypsochromic shift*) for Pd(II) and the absent of the reflection band at 252 nm in the two mentioned complexes attributed to complexation behavior of norfloxacin towards metal ions.

Table 2. Infrared frequencies[a] (cm^{-1}) and tentative assignments[b] for Norfloxacin (NOR); $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ and $[Pd(NOR)_2]Cl_2.3H_2O$.

NOR	$[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$	$[Pd(NOR)_2]Cl_2.3H_2O$	Assignment
3425w	3366m,br	3434m,br	ν(O-H);H_2O , COOH
3332vw	-	-	ν(N-H)
3046ms	2972ms	2969w,sh	ν(C-H)
2942vs 2915vw 2828vw 2728m 2554ms 2364w	2819w 2487ms 2361ms,sh	2840w 2772vw 2552m 2361ms,sh	ν(-NH_2^+)
1729vs	-	-	ν(C=O); COOH
-	1630vs	1621vs	$\nu_{as}(COO^-)$
1619vs 1586vs 1521w	1569s 1520vw	1583s 1500vw	ν(C=O) and phenyl breathing modes
1479s 1443w 1406vw	1491vs, sh	1481s, sh	-CH; deformations of -CH_2
-	1403ms	1383s,sh	$\nu_s(COO^-)$
1376ms 1303vw	1346m	1345s	δ_b(-CH_2)
1250vs 1204w 1143vw	1271vs 1190s 1141m	1268vs 1183m 1134w	ν(C-C) ν(C-O) ν(C-N)
1124vw 1097s 1030ms	1116vw 1086ms 1039s	1097m 1029ms	δ_r(-CH_2)
937vs,sh 885ms 833s	931s,sh 819s	931s 825ms	-CH bend; phenyl
798vw 782w 747ms 700m	747w 701vw	779vw 743m 700vw	$\delta_b(COO^-)$
660w 621m 561m 516vw 505vw 449m	663vw 624m 565w 514w 414vw	660w 622m 557vw 520vw,sh 421vw	ν(M-O)+ring deformation

[a]s=strong, w=weak, sh=shoulder, v=very, br=broad, [b]ν=stretching, δ=bending.

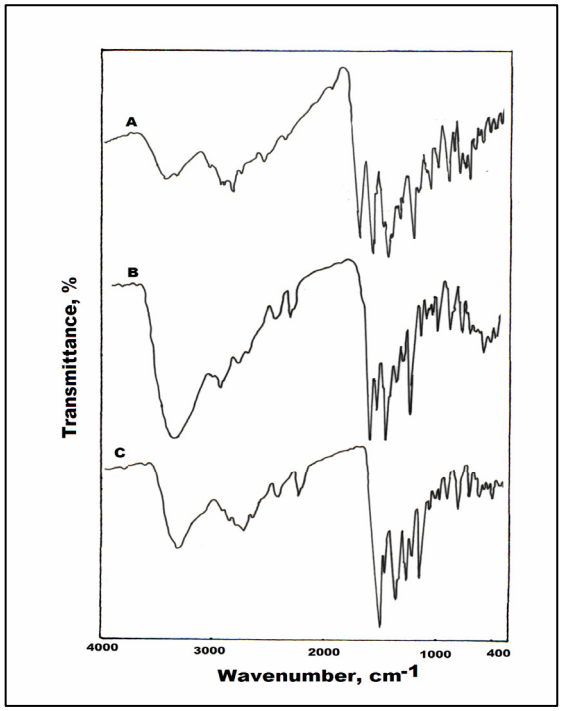


Figure 1. Infrared spectra of (A): NOR;
(B): $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$;
(C): $[Pd(NOR)_2]Cl_2.3H_2O$ complexes

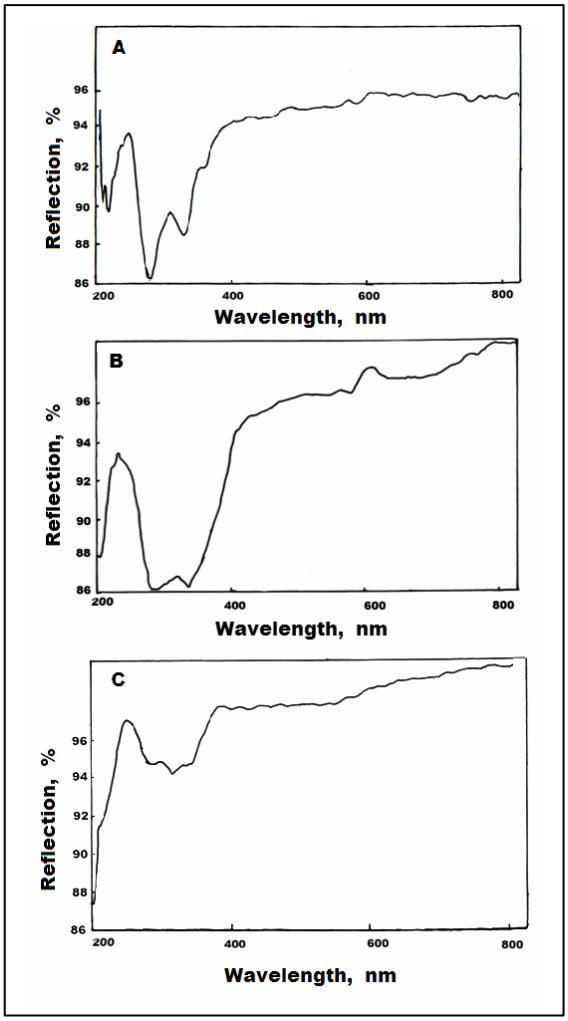


Figure 2. Electronic reflection spectra of
(A): NOR;
(B): $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$;
(C): $[Pd(NOR)_2]Cl_2.3H_2O$ complexes**.**

Thermal analyses

Thermogravimetric (TG) and differential thermogravimetric (DrTGA) analyses were carried out for norfloxacin, Y(III) and Pd(II) norfloxacin complexes under a N_2 flow, Figure 3 and Table 3, gives the maximum temperature values for decomposition along with the corresponding weight loss values. The data obtained indicate that the norfloxacin is thermally stable in the temperature range 25-50 ^oC. Decomposition of the NOR start at 50 ^oC and finished at 726 ^oC with two stages. The first stage of decomposition occurs at maximum temperature of 116 ^oC and is accompanied by a weight loss of 8.75%, corresponding exactly to the loss of ethylene molecule (C_2H_4). The second stage of decomposition occurs at three maxima 330, 423 and 654 ^oC and is accompanied by a weight loss of 83.73%, corresponding to the loss of $6C_2H_2$+3NO+HF+0.5H_2. The actual weight loss from these stages is equal to 92.48%, very closer to calculated value 92.46% [3-5].

The thermal decomposition of $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ complex in inert atmosphere proceeds approximately with two main degradation steps. The first step of decomposition occurs at maximum temperature of 60 ^oC and is accompanied by a weight loss of 13.70%, corresponding to the loss of eight water molecules. The second stage of decomposition occurs at maximum temperature of 320 and 522 ^oC. The weight loss at this step is 69.850%, corresponding to the loss of $8C_2H_2$+$5C_2H_4$+3HCl+2HF+CO+6NO+1.5H_2O as will be described by the mechanism of the decomposition, then final thermal product obtained at 800 ^oC is $YO_{1.5}$+5C.

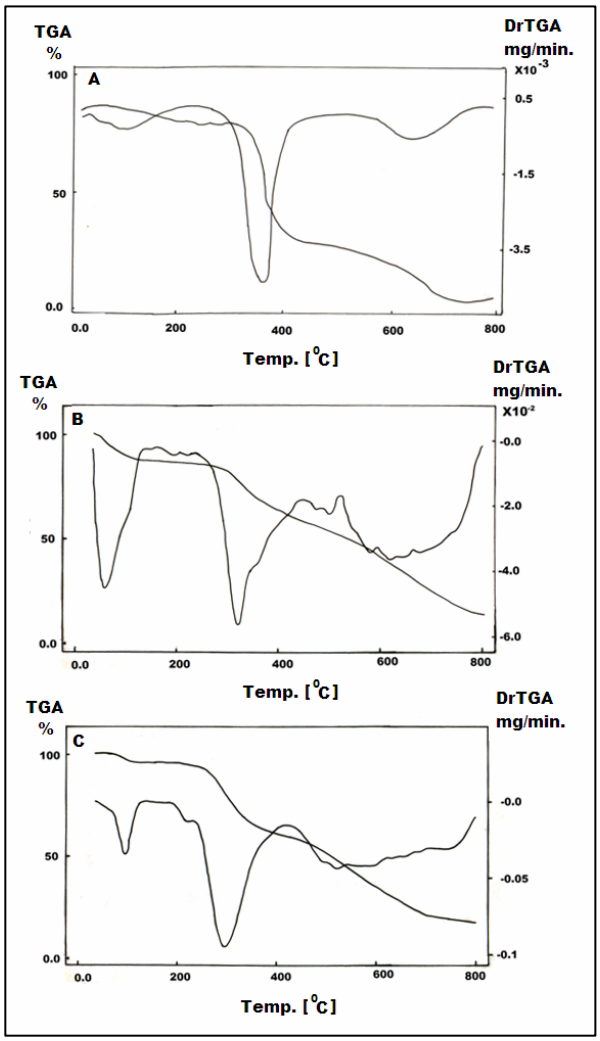


Figure 3. TGA and DrTG diagrams of (A): NOR; (B): $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$; (C) $[Pd(NOR)_2]Cl_2.3H_2O$ complexes**.**

Table 3. The maximum temperature T_{max}/°C and weight loss values of the decomposition stages for Y(III) and Pd(II) norfloxacins.

Compounds	Decomposition Steps	Tmax/°C	Weight loss (%)	
			Calc.	Found
NOR ($C_{16}H_{18}N_3O_3F$)	1st 2nd Total loss, Residue	116 330, 423, 654	8.77 83.69 92.46, 7.54	8.75 83.73 92.48, 7.52
$[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ ($C_{32}H_{60}N_6O_{18}F_2Cl_3Y$)	1st 2nd Total loss, Residue	60 320, 522	13.722 69.802 83.524, 16.476	13.70 69.850 83.55, 16.45
$[Pd(NOR)_2]Cl_2.3H_2O$ ($C_{32}H_{42}N_6O_9F_2Cl_2Pd$)	1st 2nd Total loss, Residue	94 295, 522	6.211 76.030 82.241, 17.759	6.200 76.084 82.284, 17.716

Hydrated Pd(II) norfloxacin complex loss upon heating three water molecules in the first stage at maximum temperature 94 °C. The second step of decomposition occurs at two maxima temperature at 295 and 522 °C. This step is associated with the loss of norfloxacin forming Pd+4C as a final product. The dehydration process for Pd(II) complex takes place in a single step. The single step of dehydration indicates that only lattice water is included in the structure of this compound. On the other hand the dehydration process for Y(III) complex show two steps, which indicate that the presence of coordinated water in the structure of this complex, which was also confirmed by the DFT- data (B3LYP/CEP-31G). The found weight loss associated with each step of decomposition for our complexes agree well with the calculated weight loss, Table 3. The final products of two complexes obtained at 800 °C were confirmed with infrared spectra.

Kinetic studies

The kinetic parameters for norfloxacin ligand and the two complexes have been evaluated from TGA curves by using Coats-Redfern (CR) integral method and linearization method of Horowitz and Metzger (HM) [24,25]. The values of kinetic parameters are shown in Figure 4 and Table 4 and the results obtained by these methods are compared with one another. The entropy of activation ΔS^* in (J K^{-1} mol^{-1}) was calculated by using the equation: $\Delta S^*=R \ln(Ah/k_BT_s)$, where k_B is the Boltzmann constant, h is the Plank's constant, R is the gas constant in (J mol^{-1} K^{-1}) and T_s is the DTG peak temperature [5,26]. The enthalpy activation, ΔH^*, and Gibbs free energy, ΔG^*, were calculated from; $\Delta H^*=E^*-RT$ and $\Delta G^*=\Delta H^*-T\Delta S^*$, respectively. The energy of activation is 19.1-3.077×10^4 J mol^{-1}.

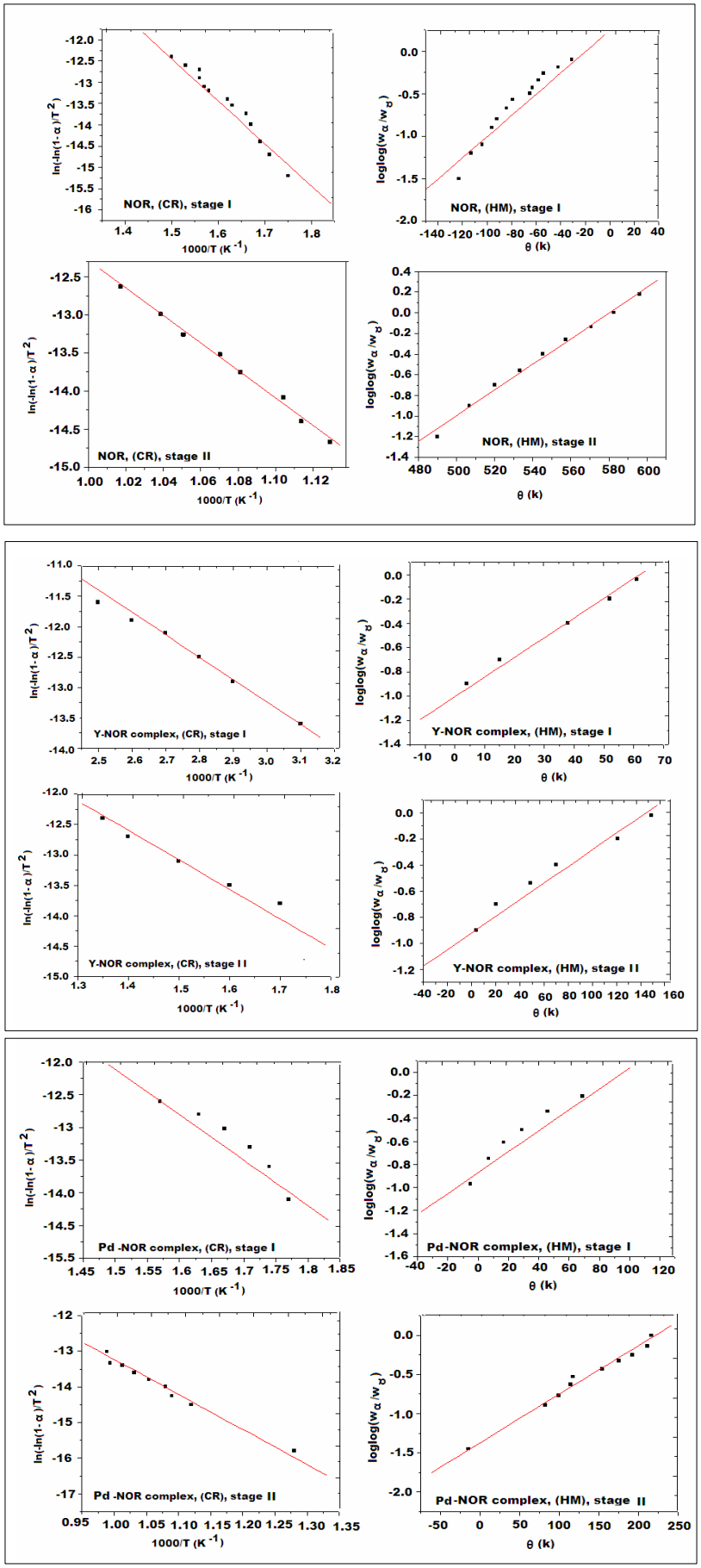


Figure 4. The diagrams of kinetic parameters of (A): NOR; (B):$[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$; (C): $[Pd(NOR)_2]Cl_2.3H_2O$ complexes**.** using Coats-Redfern (CR) and Horowitz.-Metzger (HM) equations**.**

Table 4. Thermal behavior and kinetic parameters determined using the Coats–Redfern (CR) and Horowitz–Metzger (HM) operated for norfloxacin and their complexes.

Complexes	Decomposition Range (K)	T_s(K)	Method	Parameter					R
				$E^*\times10^4$ (J mol^{-1})	A (s^{-1})	$\Delta S^*\times10^2$ (J mol^{-1} K^{-1})	$\Delta H^*\times10^4$ (J mol^{-1})	$\Delta G^*\times10^5$ (J mol^{-1})	
NOR	537-734	696	CR	8.295	5.944×10^5	-1.958	7.716	2.135	0.9216
			HM	11.585	6.838×10^7	-1.020	11.006	1.810	0.8661
	844-996	927	CR	17.5	16.7×10^3	-1.74	16.7	3.28	0.9945
			HM	19.1	3.82×10^5	-0.9	18.3	2.67	0.9948
$[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$	316-444	333	CR	3.077	2.033×10^3	-1.825	2.800	0.887	0.9548
			HM	3.444	4.452×10^4	-1.568	3.167	0.839	0.9717
	570-815	593	CR	4.040	0.070×10^3	-2.153	3.547	1.634	0.9079
			HM	4.296	0.422×10^3	-2.004	3.803	1.568	0.9582
$[Pd(NOR)_2]Cl_2.3H_2O$	527-727	568	CR	5.792	6.257×10^3	-1.776	5.320	1.541	0.8699
			HM	5.621	14.61×10^3	-1.705	5.149	1.484	0.9028
	759-1027	795	CR	8.196	1.606×10^3	-1.917	7.535	2.278	0.8795
			HM	7.503	5.731×10^3	-1.811	6.842	2.124	0.91732

^{1}HNMR spectra

The formation of the metal complexes was confirmed by ^{1}HNMR spectra. Figure 5 represents the ^{1}HNMR spectrum of $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ complex which was carried out in DMSO-d_6 as solvent. Upon comparison with the free ligand the absent of the characteristic peak for H(COOH) at δ 11.00 ppm in this complex indicates coordination of NOR ligand to Y(III) through the deprotonated carboxylic group [5]. The spectrum also show characteristic peaks for quaternary nitrogen ($-^+NH_2$) at δ 2.500 and 2.494 ppm for Y(III) complex. The peak characteristic for water molecules was observed at δ 3.546, 3.383 and 3.266 ppm, which not found in the free norfloxacin. The ^{1}HNMR data for free NOR and this complex are summarized in Table 5 and all assignments are given.

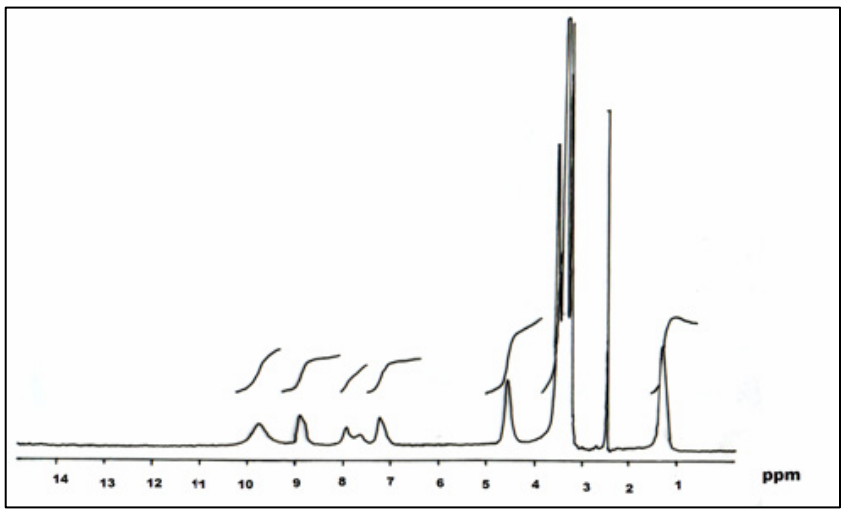


Figure 5. ^{1}H-NMR spectrum of $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ complex in DMSO, δ_{TMS}.

Table 5. ^{1}HNMR values (ppm) and tentative assignments for NOR (A) and $[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$ complex (B).

A	B	Assignments
1.13	1.223, 1.360	δ H, $-CH_3$
2.0	-	δ H, -NH; piperazine
-	2.494, 2.500	δ H, $-^{+}NH_2$
-	3.266, 3.383, 3.546	δ H, H_2O
2.78, 3.10, 3.47	4.574	δ H, $-CH_2$ aliphatic
5.93, 7.12, 8.01	7.237, 7.642, 7.949	δ H, $-CH_2$ aromatic
11.00	-	δ H, -COOH

Antibacterial investigation

The antibacterial activity of the compounds, ligand and complexes, was studied against three bacterial species, such as *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* and antifungal screening was studied against two species (*penicillium (P. rotatum)* and *trichoderma (T. sp.)*). Screening was performed by determining the inhibition zone diameter values (mm) of the novel investigated compounds against microorganisms and the results obtained are tabulated in Table 6. A comparative study of ligand and their metal complexes showed that the Y(III) complex exhibit higher antibacterial activity than uncomplexed ligand but Pd(II) complex exhibit higher antibacterial activity only for Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacterial species and no antifungal activity observed for ligand and their metal complexes. The results are promising compared with the previous studies [27-29]. 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 [28]. 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.

Table 6. The inhibition diameter zone values (mm) for NOR and its compounds.

Compound	Microbial species				
	Bacteria			Fungi	
	E. coli	*P. aeruginosa*	*S. aureus*	*P. rotatum*	*T. sp*
NOR	25	13	12	0	0
$[Y(NOR)_2(H_2O)_2]Cl_3.10H_2O$	39	47	31	0	0
$[Pd(NOR)_2]Cl_2.3H_2O$	26	28	27	0	0
Control (DMSO)	0	0	0	0	0

Computational details

Computational method

The geometric parameters and energies were computed by Density Functional Theory at the B3LYP/CEP-31G level of theory, using the GAUSSIAN 98W package of the programs, on geometries that were optimized at CEP-31G basis set. The high basis set which was chosen to detect the energies has a publishable quality. By the natural atomic orbital populations, we computed the atomic charges. The B3LYP is the keyword for the hybrid functional [30], which is a linear combination of the gradient functionals proposed by Becke [31] and Lee, Yang and Parr [32], together with the Hartree-Fock local exchange function [33].

Structural parameters and models

Norfloxacin

The biological activity of quinolones, norfloxacin, is mainly determined by its fine structure, the norfloxacin has many characteristic structural features. The molecule is a highly sterically-hindered, the piperazine group out of plane of the molecule. This observation is supported by the values of calculated dihedral angles: C15-N14-C12-C13, -106.19˚, and C19-N14-C12-C13, 104.13˚ while the dihedral angle C19-N14-C12-C11, -75.58˚ where the values are neither zero nor 180˚. Scheme II shows the optimized structure of norfloxacin molecule, the dihedral angles O21-C3-C2-C1 is 5.01˚ and O23-C1-C2-C3 is 12.66˚ which confirms a cis configuration of O21 and O23 while O22-C1-C2-C3 is -169.44˚ which indicates that O21 and O23 are in cis configuration and C3-O21 in the same plane of C1-O23 while, O22 and O21 are in trans configuration and C3-O21 not in the same plane of C1-O22.

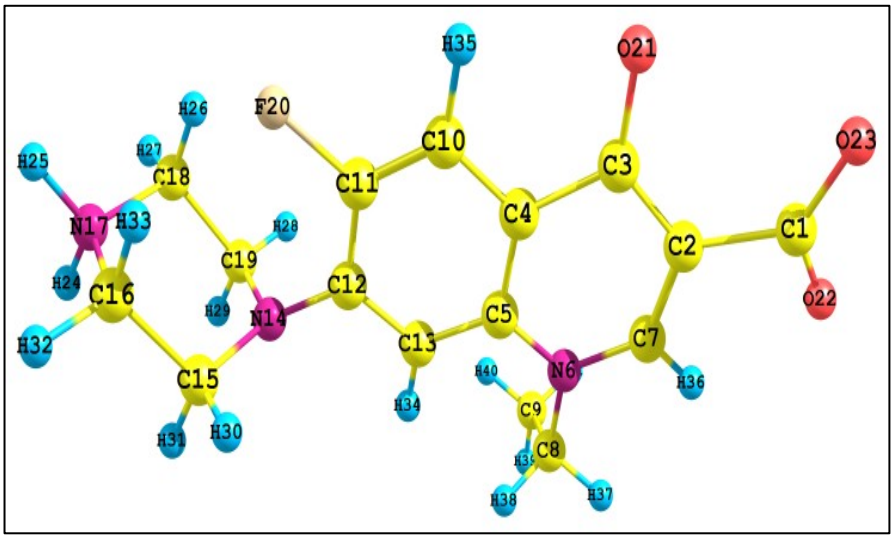
Table 7 gives the optimized geometry of norfloxacin as obtained from B3LYP/CEP-31G calculations. These data are drawing to give the optimized geometry of molecule. The bond distance of C1-O23 is 1.28Å and C1-O22 is 1.30Å while, C3-O21 is 1.27Å. The value of bond angle C3-C2-C1 is 126.99˚ reflects on sp^2 hybridization of C2, the same result is obtained with C3 and C1. The values of bond distances are compared nicely with that obtained from X-ray data [34].

Charge distribution on the optimized geometry of norfloxacin is given in Table 7. There is a significant built up of charge density on the oxygen atoms which distributed over all molecule so norfloxacin molecule behaves as bi-dentate ligand (O_{keto} and $O_{carboxilic}$) and the molecule is a highly dipole μ = 42.86D.

Table 7. Equilibrium geometric parameters bond lengths (Å), bond angles (˚), dihedral angles (˚) and charge density of norfloxacin ligand by using DFT/B3LYP/CEP-31G.

Bond length (Å)			
C3-O21	1.27 (1.30)[34]	C1-C2	1.58 (1.47)[34]
C2-C3	1.47 (1.41)	C1-O22	1.30 (1.29)
C2-C7	1.38 (1.38)	C1-O23	1.28 (1.23)
Bond angle (˚)			
C12-N14-C15	119.86	O21-C3-C2	128.17
C12-N14-C19	119.51	O22-C1-C2	113.13
N14-C12-C11	122.73	O23-C1-C2	116.91
N14-C12-C13	119.79	O23-C1-O22	129.92
C3-C2-C1	126.99		
Dihedral angles (˚)			
C16-C15-N14-C12	-95.43	C19-N14-C12-C11	-75.59
C12-N14-C19-C18	95.24	C19-N14-C12-C13	104.13
C15-N14-C12-C11	74.09	O23-C1-C2-C3	12.66
N14-C12-C11-C10	179.98	O22-C1-C2-C3	-169.44
N14-C12-C13-C5	-179.30	O21-C3-C2-C1	5.01
C15-N14-C12-C13	-106.19		
Charges			
N17	0.257	O21	-0.097
N14	-0.117	O22	-0.340
N6	0.213	O23	-0.250
C3	-0.269	C1	-0.141
Total energy/au		-202.66371	
Total dipole moment/D		42.86	

( ): Ref. [34]



Scheme II. The optimized structure of norfloxacin by using B3LYP/CEP-31G.

Table 8. Equilibrium geometric parameters bond lengths (Å), bond angles (˚) and charge density of $[Y(NOR)_2Cl_2]^+$ by using DFT/B3LYP/CEP-31G.

Bond length (Å)			
Y-O21a	2.319 (2.307) [37]	C1b-O22b	1.275 (1.245) [36]
Y-O23a	2.229 (2.207) [37]	C3b-O21b	1.289 (1.275) [36]
Y-O21b	2.315 (1.307) [37]	C1a-O23a	1.312 (1.278) [36]
Y-O23b	2.229 (2.207) [37]	C1a-O22a	1.275 (1.245) [36]
Y-Cla	2.666 (2.56) [38]	C3a-O21a	1.291 (1.275) [36]
Y-Clb	2.671 (2.69) [38]	C1b-O23b	1.312 (1.278) [36]
Bond angle (˚)			
O21b-Y-O23b	76.64 (81.30) [35]	O21a-Y-O23b	97.59 (91.5) [35]
O21b-Y-O23a	109.57 (91.50) [35]	O21a-Y-Cla	95.59
O21b-Y-Cla	85.76	O21a-Y-Clb	90.19
O21b-Y-Clb	88.67	O23a-Y-Cla	90.97
O21a-Y-O-23a	76.18 (82.50) [35]	O23a-Y-Clb	88.56
O23b-Y-Cla	90.28	O23b-Y-Clb	90.82
Charges			
Y	1.082	Cla	-0.444
O23a	-0.294	Clb	-0.446
O21a	-0.162	O23b	-0.298
O22a	-0.167	O21b	-0.156
O22b	-0.168		
Total energy/au		-473.6626	
Total dipole moment/D		4.33	

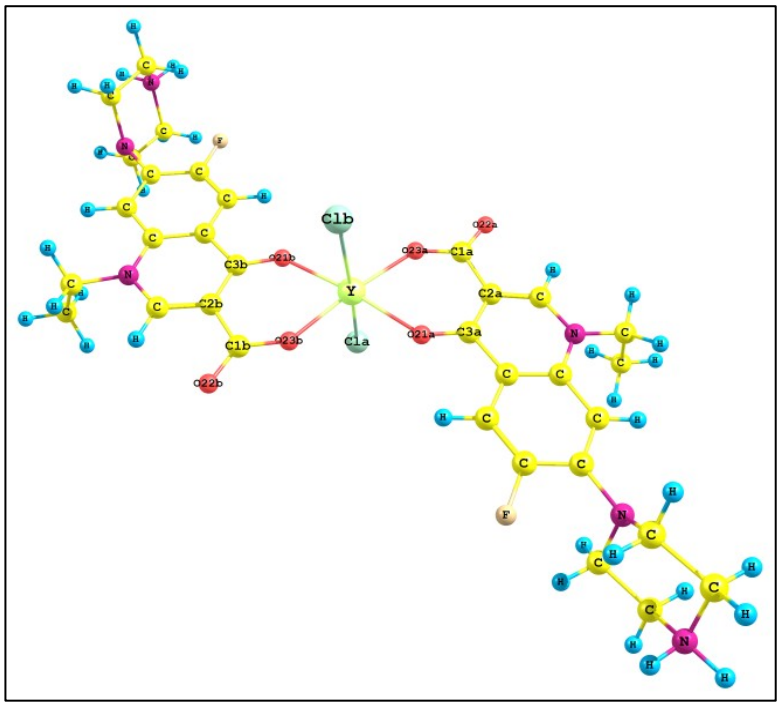
Yttrium-norfloxacin complex

The Y(III) chelated with two molecules of norfloxacin through the oxygen atom of the carboxylate group and the oxygen atom of the carbonyl group forming four bonds. The experimental data set that the result complex is six-coordinate so, the complex consists of four coordinate bonds with two norfloxacin molecules and there are other two coordinated bonds may be with water molecules or chloride ions. In this part we study theoretically the two structures $[Y(NOR)_2Cl_2]^+$ and $[Y(NOR)_2(H_2O)_2]^{3+}$ in order to determinate the structure which is more stable.

Description of the structure of $[Y(NOR)_2Cl_2]^+$

Scheme III shows the optimized structure of the complex with the atomic numbering scheme selected bond distances and angles are given in Table 8. The suggested complex is composed of $[Y(NOR)_2Cl_2]^+$, where the Yttrium ion is in a distorted octahedral environment. In the equatorial plane the metal ion is coordinated by four oxygen atoms (O_{keto} and $O_{carboxylic}$) of two norfloxacin ligands at the distances vary from 2.229Å to 2.319Å, these bond lengths are similar to those observed in related compounds [35]. The difference in the carboxylate O23a-C1a and O22a-C1a (1.312Å and 1.275Å) [36], confirms the formation of bond between the ionic carboxylate oxygen atom and Yttrium ion; these bond lengths are virtually identical in uncomplexed norfloxacin ligand. The octahedral coordination environment is completed by two chloride ions. The bond distance between Y-O23a is 2.229Å [36] and Y-O21a is 2.319Å [37] while the distance between Y-Cl is 2.671Å [38]. The bond angles around the central metal ion Y(III) vary from 76.64˚ to 109.57˚; these values differ significantly from these expected for a regular octahedron.

The distances and angles within the ligand moiety are similar to those described for free norfloxacin [34]. It is important to note that, in the complex, the C1a-O23a and C3a-O21a bond lengths become equal 1.312 and 1.291Å which are slightly longer than those found in the free norfloxacin 1.278 and 1.275Å while the dipole moment is weak and equal to 4.33D, sothat this complex is less stable.



Scheme III. Optimized structure of $[Y(NOR)_2Cl_2]^+$ complex by using B3LYP/CEP-31G.

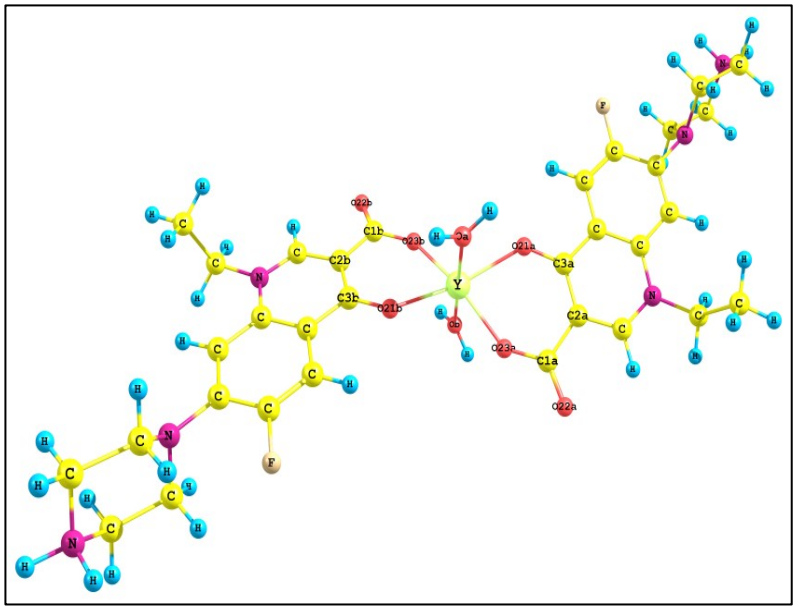
Description of the structure of $[Y(NOR)_2(H_2O)_2]^{3+}$

Table 9 lists selected inter atomic distances and angles. The structure of complex with atomic numbering scheme is shown in Scheme IV. The complex consists of two units of norfloxacin molecule and two water molecules with metal ion Y(III). The complex is six-coordinate with distorted octahedral environment around the metal ion. The Y(III) is coordinated to one oxygen atom of the carbonyl group and one oxygen atom of the carboxylate group for each norfloxacin ligand and two oxygen atoms for two water molecules. The Y-O21a and Y-O21b bond lengths are 2.283 and 2.283Å which become longer than that Y-O23a and Y-O23b, 2.167 and 2.167Å. Both are similar to these observed in related quinolone Y(III) compounds [37]. Also the angles around the central metal ion Y(III) with surrounding oxygen atoms vary from 77.13˚ to 107.13˚; these values differ legally from these expected for a regular octahedron. The distances and angles in the quinolone ring system, as well as those of piprazine rings are similar to those found in reported structure of free norfloxacin and norfloxacin compounds [34].

Table 9. Equilibrium geometric parameters bond lengths (Å), bond angles (˚) and charge density of $[Y(NOR)_2(H_2O)]^{3+}$ by using DFT/B3LYP/CEP-31G.

Bond length (Å)			
Y-O21a	2.283 (2.307) [37]	C1b-O22b	1.261 (1.245) [36]
Y-O23a	2.167 (2.207) [37]	C3b-O21b	1.321 (1.275) [36]
Y-O21b	2.283 (1.307) [37]	C1a-O23a	1.339 (1.278)[[36]
Y-O23b	2.167 (2.207) [37]	C1a-O22a	1.261 (1.245) [36]
Y-Oa	2.356 (2.390) [37]	C3a-O21a	1.321 (1.275) [36]
Y-Ob	2.368 (2.388) [37]	C1b-O23b	1.339 (1.278) [36]
Bond angle (˚)			
O21b-Y-O23b	77.13 (81.30) [35]	O21a-Y-O23b	107.59 (91.5) [35]
O21b-Y-O23a	107.13 (91.50) [35]	O21a-Y-Oa	101.13
O21b-Y-Oa	101.15	O21a-Y-Ob	78.88
O21b-Y-Ob	78.83	O23a-Y-Oa	77.97
O21a-Y-O23a	77.14 (82.50) [35]	O23a-Y-Ob	102.04
O23b-Y-Oa	77.96	O23b-Y-Ob	102.03
Charges			
Y	1.668	Oa	0.145
O23a	-0.372	Ob	0.132
O21a	-0.256	O23b	-0.372
O22a	-0.084	O21b	-0.256
O22b	-0.085		
Total energy/au		-477.627597	
Total dipole moment/D		7.175	

The bond distances between Y(III) and surrounded oxygen atoms of norfloxacin in water complex are shorter than that in chloride complex as shown in Tables 7 and 8, so that the metal ion Y(III) is bonded strongly with surrounded oxygen atoms of norfloxacin in water complex more than that in chloride complex. Also the charge accumulated on oxygen atom of the carboxylate group and the oxygen atom of the carbonyl group in water complex are -0.372and -0.256, respectively, and the complex is highly dipole 7.175D , while in the case of chloride complex are -0.294 and -0.162, respectively. There is a strong interaction between central metal ion Y(III) which become has charge equal +1.66 and more negative oxygen atoms in water complex greater than that in chloride complex, at which Y(III) becomes has less positively charge (+1.08) in chloride complex. For all these reasons the water complex is more stable than chloride complex and Y(III) favor coordinated with two molecules of water more than two chloride ions to complete the octahedron structure.



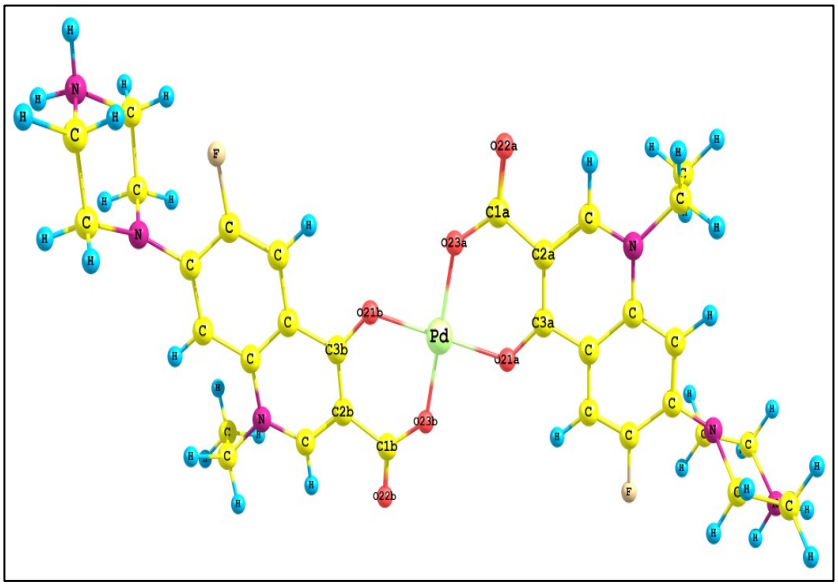
Scheme IV. Optimized structure of $[Y(NOR)(H_2O)_2]^{3+}$ complex by using B3LYP/CEP-31G.

Palladium-norfloxacin complex

Table 10 lists selected inter atomic distances and angles. The structure of complex with atomic numbering scheme is shown in Scheme V. The complex is four-coordinate, the metal ion Pd (II) is coordinated to two unites of norfloxacin molecules, one O_{keto} atom and one $O_{carboxylate}$ atom of each norfloxacin ligand. The Pd-O_{keto} bond lengths (2.03Å and 2.029Å) are longer than the Pd-$O_{carboxylate}$ (2.019Å and 2.019Å). Both are similar to these observed in related quinolone Pd(II) complex [39,40]. The angles around central metal ion Pd(II) with surrounded four oxygen atoms of norfloxacin vary from 87.76˚ to 92.26˚, these values not deviated slightly from these expected for square planar. The distances between Pd-$O_{carboxylate}$ are (2.019Å and 2.019Å) [39] and Pd-O_{keto} are (2.029Å and 2.030Å) [39], these values are agreement nicely with experimental data [39,40].

Table 10. Equilibrium geometric parameters bond lengths (Å), bond angles (˚) and charge density of $[Pd(NOR)_2]^{2+}$ by using DFT/B3LYP/CEP-31G.

Bond length (Å)			
Pd-O21a	2.030 (1.990)[39]	C1b-O22b	1.269 (1.245)[36]
Pd -O23a	2.019 (2.009)[40]	C3b-O21b	1.304 (1.275)[36]
Pd –O21b	2.029 (2.006)[39]	C1a-O23a	1.329 (1.278)[36]
Pd -O23b	2.019 (2.009)[40]	C1a-O22a	1.269 (1.245)[36]
C1b-O23b	1.329 (1.278)[36]	C3a-O21a	1.303 (1.275)[36]
Bond angle (˚)			
O21b -Pd -O23b	92.26 (95.76)[39]	O21a -Pd -O23b	87.71
O21b –Pd- O23a	87.76	O21a –Pd- O23a	92.26 (95.76)[39]
Charges			
Pd	0.614	O23b	-0.278
O23a	-0.278	O21b	-0.242
O21a	-0.239	O22b	-0.204
O22a	-0.203		
Total energy/au		-532.490724	
Total dipole moment/D		4.71	



Scheme V. Optimized structure of $[Pd(NOR)_2]^{2+}$ complex by using B3LYP/CEP-31G.

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