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Norfloxacin Antibiotic Drug Complexes of (I), (II), (III) and (IV) Metal Ions: Spectroscopic and Biological Studies.

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ABSTRACT

The new norfloxacin (nor) complexes of Li(I), Pb(II), Sb(III) and Se(IV) with general formulas: [Li(nor)].X (where X= Cl, NO_3 or CH_3COO), $[Pb(nor)_2].X_2$ (where X= Cl or NO_3), $[Sb(nor)_3].Cl_3$ and $[SeO_2(nor)_2]$ have been synthesised and characterized with the help of (infrared and ^{1}H-NMR) spectroscopic, molar conductivity and elemental analysis tools. The antimicrobial activities of complexes were tested against some kind of bacteria and fungi.

Keywords: Infrared spectra; ^{1}H-NMR, norfloxacin; antimicrobial activities.

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INTRODUCTION

Quinolones drugs are namely as quinolonecarboxylic acids or 4-quinolones, which are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton [1, 2]. The fluoroquinolones are very active against aerobic Gram-negative microorganisms but less active against Gram-positive microorganisms [1, 3]. They are extremely useful for the treatment of a variety of infections, including urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community acquired pneumonia, acute bronchitis and sinusitis [1-5]. Fluoroquinolones antibiotics have a ketone at position 4 and a carboxylic group at position 3. Fluoroquinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme. Fluorine at position 6 enhances gyrase inhibition and cell penetration. Piperaziny substituents provide activity against Gram-negative bacteria and pyrrolidinyl moiety is active against Gram-positive cocci. They improve water solubility needed oral application. The function substituted at position 8 is to control anaerobe activity. The uptake of norfloxacin (nor; Scheme 1) by Escherichia coli has been investigated at different pH and monovalent/divalent metal ion concentrations [6]. The results of the study supported a simple diffusion mechanism for quinolone incorporation into cells. The uptake process decreases under acidic conditions. The presence of Na^+ and K^+ ions does not affect the results to an appreciable extent, whereas divalent ions cause a dramatic decrease in drug incorporation. The antibacterial activity evaluated under identical experimental conditions shows a direct relationship with the uptake data. It was suggested that the ability of the drug to penetrate into cells is a function of its net charge. The molecule in zwitterionic form exhibits maximum permeation properties, whereas the uptake is strongly reduced when the drug bears a net charge as a result of ionization or complex formation with divalent ions. The proposed mechanism of the interaction between quinolone and metal cations was chelation between the metal and the 4-oxo and adjacent carboxyl groups. Since these functional groups are required for antibacterial activity, it could be anticipated that all of the quinolones will interact with metal ions. However, there may be differences between the quinolones regarding the extent of interaction [7]. The crystal structures of several free quinolone molecules have been determined: nalidixic acid [8, 9], pefloxacin methanesulfonate [10, 11], cinoxacin [12], norfloxacin (nor) [13, 14], ciprofloxacin hexahydrate [15], ciprofloxacin lactate [16], norfloxacin dihydrochloride [17, 18]. It is interesting to note that in most cases the carboxylic group is not deprotonated and the hydrogen atom of this group is hydrogen bonded to an adjacent 4-oxo atom. In a few examples [13-15], the carboxylic group is ionized and the molecule thus exists in a zwitterionic form with protonated terminal nitrogen of the piperazine ring in a solid state. A complex of magnesium(II) with the formula $[Mg_2(H_2O)_6(nfH)_2]Cl_4$ was isolated by hydrothermal reaction [19]. It can be described as 2:2 dimer in which the two magnesium ions are bridged by two oxygen atoms from carboxylate groups of the two norfloxacin molecules. The coordination mode of carboxylate can be considered as a monodentate bridging type. A calcium complex, $[Ca_2(Cl)(nfH)_6]Cl_3$ was also isolated by hydrothermal reaction [19]. This complex is also a dimer, but the bridging group is a chloride ion. The coordination geometry around each calcium ion can best be described as a distorted pentagonal bipyramid. Four chloride ions are coordinated to a copper(II) ion, in forming the rather distorted tetrahedron in (nfH3)(nfH2)$[CuCl_4]$Cl [18]. There are two nonequivalent norfloxacin molecules in the asymmetric unit. Also the zinc compound (nfH3)(nfH2)$[ZnCl_4]$Cl is isotypical to the copper/norfloxacin compound. The goal of this paper is to synthesis and characterization of new metal complexes with norfloxacin antibiotic drug which have antibacterial agents. The objective of this study is the isolation and characterization of the Li(I), Pb(II), Sb(III) and Se(IV) norfloxacin complexes, as well as their characterization using spectroscopic analyses. The antibacterial activity of the investigated complexes was tested against *Escherichia Coli* (Gram -ve), *Bacillus subtilis* (Gram +ve) and antifungal activity was also investigated (tricoderma and penicillium activities).

EXPERIMENTAL

Chemicals

All chemicals used for the preparation of the complexes were of analytical reagent grade, commercially available, used without further purification and received from different sources (Fluka and Aldrich).

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Synthesis of norfloxacin metal complexes

All the nor complexes were prepared as follows, employing a 1:1, 1:2 and 1:3 (metal ions: nor) ratios for the Li(I), (Pb(II) and Se(IV)) and Sb(III) metal ions, respectively. A solution of 1.0 mmol of each salt of (LiCl, $LiNO_3$ and CH_3COOLi), ($PbCl_2$ and $Pb(NO_3)_2$), SeO_2, and $SbCl_3$ previously dissolved in 10 mL of distilled water was added to a suspended solution of (1.0, 2.0 or 3.0) mmol of nor in 50 mL of acetone. The resulting mixtures were heated at ~ 50 ^{o}C under reflux on a water bath for about 24 h and then cooled. The obtained complexes were separated from the reaction mixture by filtration, washed with boiling water and acetone and dried under *vacuum* over $CaCl_2$.

Instrumental

The elemental analyses CHN contents were determined using a Perkin-Elmer CHN 2400. IR spectra were recorded on a Bruker FT-IR Spectrophotometer [(4000 to 400 cm^{-1})] in KBr pellets. Molar conductivities of the freshly prepared solutions with a concentration of 1.0×10^{-3} mol/L in DMSO were measured using a Jenway 4010 conductivity meter. The chloride, nitrate and acetate anions analysis was performed as follows: the complexes were dissolved in 5 mL concentrated HNO_3, and the obtained samples diluted with water to 10 mL. The qualitative analysis of the Cl^{-}, CH_3COO^{-} and NO_3^{-} ions were performed [20].

Antibacterial investigation

The procedure described by Gupta et al., [21] was employed. The investigated isolates of bacteria were seeded in tubes with nutrient broth (NB). The seeded NB (1 mL) was homogenized in the tubes with 9 mL of melted (45 ^{o}C) nutrient agar (NA). The homogeneous suspensions were poured into Petri dishes and left till solidified. Some holes were spread on the top of solidified media. Holes having a diameter of 4 mm impregnated with 2×10^{-3} dm^3 of the test. After incubation for 24 h in a thermostat at 25 ^{o}C, the inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. An inhibition zone diameter over 7 mm indicates that the tested compound is active against the bacteria under investigation. The antibacterial activities of the investigated compounds were tested against *Escherichia Coli* (Gram -ve), *Bacillus subtilis* (Gram +ve), as well as for antifungal activity (tricoderma and penicillium).

RESULTS AND DISCUSSION

The physical and elemental analysis data of norfloxacin complexes are listed in Table 1. The norfloxacin complexes have formulas [Li(nor)].X (where X= Cl, NO_3 or CH_3COO), [Pb(nor)$_2$].X_2 (where X= Cl or NO_3), [Sb(nor)$_3$].Cl_3 and [SeO_2(nor)$_2$]. The test for anions is positive which indicated the presence of them outside the coordination sphere. All norfloxacin complexes are solids, colored and freely soluble in dimethylformamide (DMF) or dimethylsulfoxide (DMSO). Conductivity measurements in dimethylsulfoxide indicated them to be highly conductive 20-to-197 $ohm^{-1}cm^2mol^{-1}$ [22-24]. All complexes were decomposed over >250 ºC indicating their thermal stability.

Table 1: Elemental analysis, molar conductance and color of the norfloxacin complexes

Complexes	(Calcd) Found				Molar conductance $\Omega^{-1}cm^2mol^{-1}$	Color
	%C	%H	%N	%M		
[Li(nor)].Cl	(53.13)53.10	(5.02)4.96	(11.62)11.54	(1.92)1.87	77	White
[Li(nor)].NO_3	(49.49)49.31	(4.67)4.63	(14.43)14.32	(1.79)1.77	82	White
[Li(nor)].CH_3COO	(56.11)55.98	(5.49)5.42	(10.91)10.79	(1.80)1.76	70	White
[Pb(nor)$_2$].Cl_2	(41.92)41.77	(3.96)3.91	(9.17)9.13	(22.60)22.35	135	Pale yellow
[Pb(nor)$_2$].$(NO_3)_2$	(39.63)39.44	(3.74)3.45	(11.55)11.32	(21.36)21.30	124	Pale yellow
[Sb(nor)$_3$].Cl_3	(48.61)48.39	(4.59)4.50	(10.63)10.41	(10.27)10.11	198	White
[SeO_2(nor)$_2$]	(51.27)51.08	(4.84)4.81	(11.21)11.09	(10.53)10.50	20	Pale yellow

Infrared spectra

Infrared spectral data of norfloxacin free drug ligand and its Li(I), (Pb(II) and Se(IV)) and Sb(III) complexes are listed in Table 2. The IR spectra of the complexes (Fig. 1-4) are compared with those of the free

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ligand in order to determine the coordination sites that may involved in chelation. There are some guide peaks, in the spectra of the ligand, which are useful in achieving this goal. The position and/or the intensities of these peaks are expected to be changed upon chelation. These guide peaks are listed in Table 2. The *v*(OH), *v*(C=O), v_{asym}(COO) and v_{sym}(COO) stretching vibrations are observed at (3448, 1727, 1590 and 1396) cm^{-1} for the free ligand. The participation of the carboxylate O atom in the norfloxacin–metal complexes formation is evidenced from the shift in position of this band at 1727 cm^{-1} to (1710-1729) cm^{-1} or disappears. For comparison the carbonyl-O; *v*(C=O), stretching vibration is found in the free ligand at 1716 cm^{-1} [25]. This band is shifted to lower wavenumbers (1628-to-1618) cm^{-1} in the complexes indicating the participation of the carbonyl-O in coordination. There are some new bands existed in the spectra of the complexes in the regions 571-to-460 cm^{-1}, which are assigned to *v*(M-O) stretching vibrations of carboxylate-O and carbonyl-O. The infrared spectra of all complexes show a sharp broad absorption near ~ 3400 cm^{-1} and a group of bands with different intensity in the range 2800-2700 cm^{-1} and 2500-2400 cm^{-1} [25]. These bands are assigned to the vibration of the quaternized nitrogen of the piperazinyl group which indicates that the zwitterionic form of norfloxacin (Fig. 1) is involved in the coordination to the metal ions investigated [22-24].

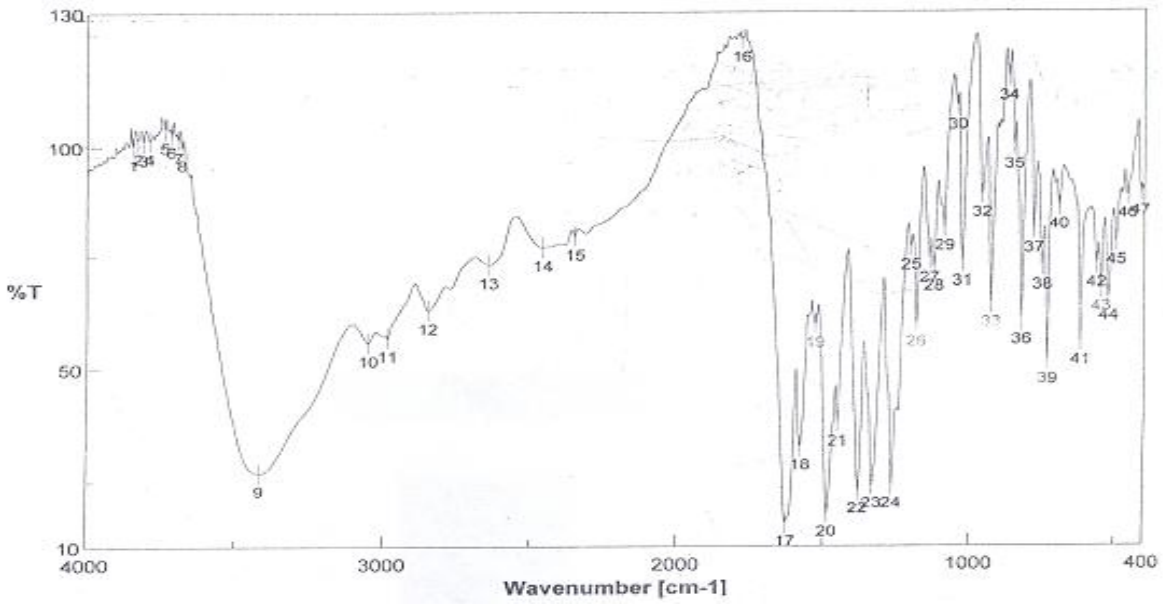

Fig. 1a

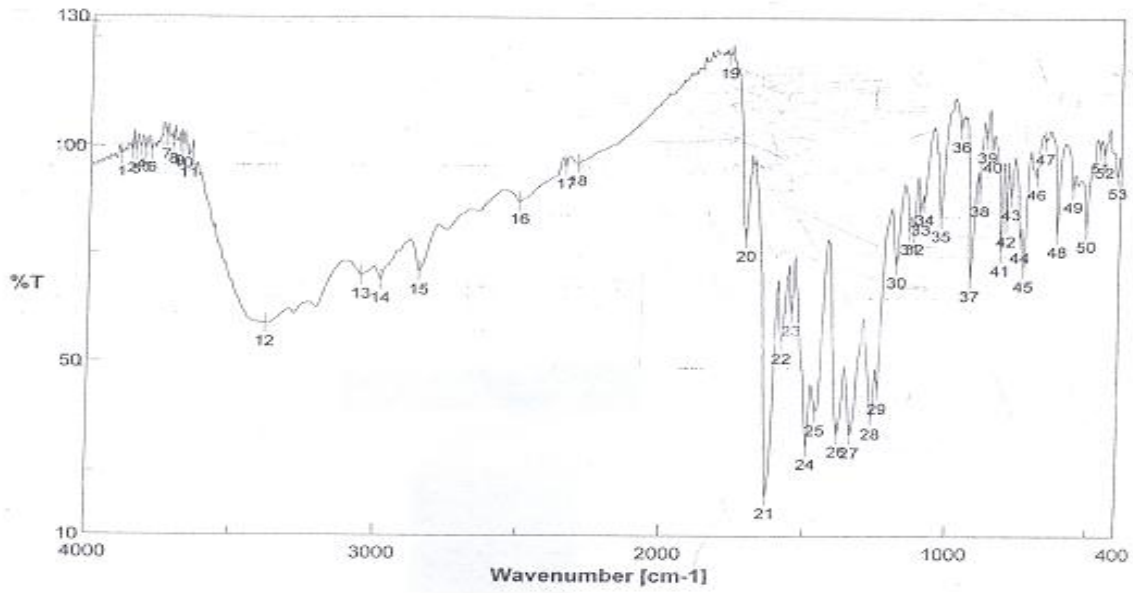

Fig. 1b

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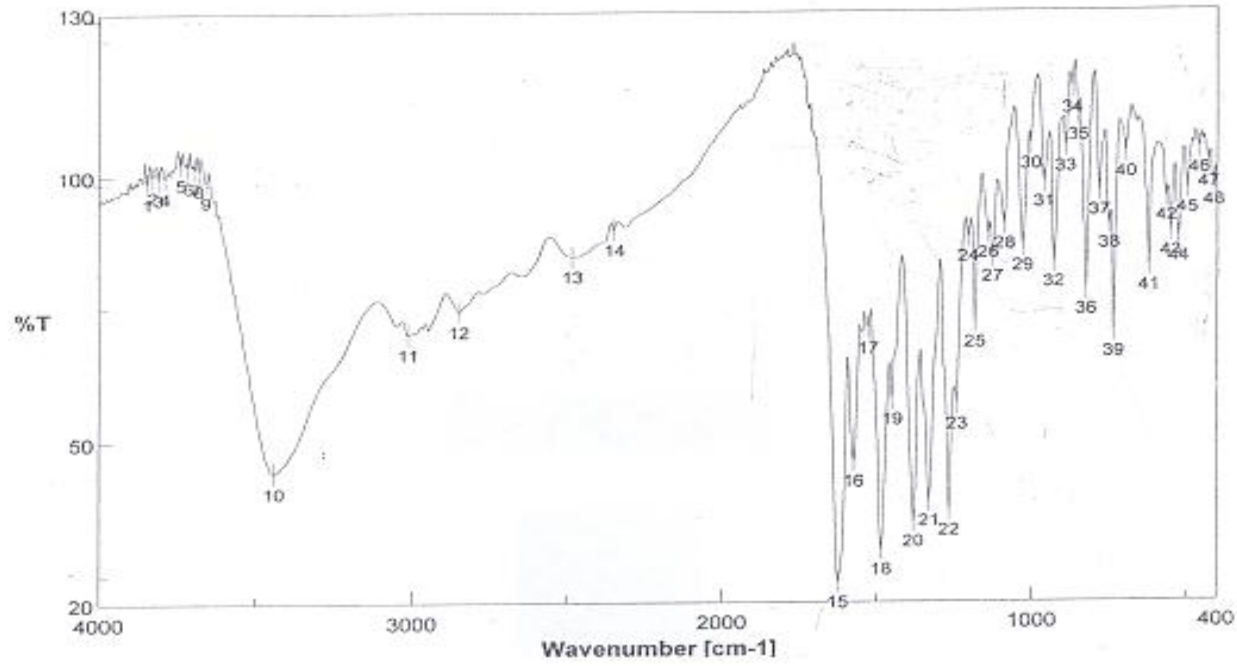

Fig. 1c

Figure 1: Infrared spectra of Li(I) norfloxacin complex (a= Cl, b= NO_3 and c= OAc)

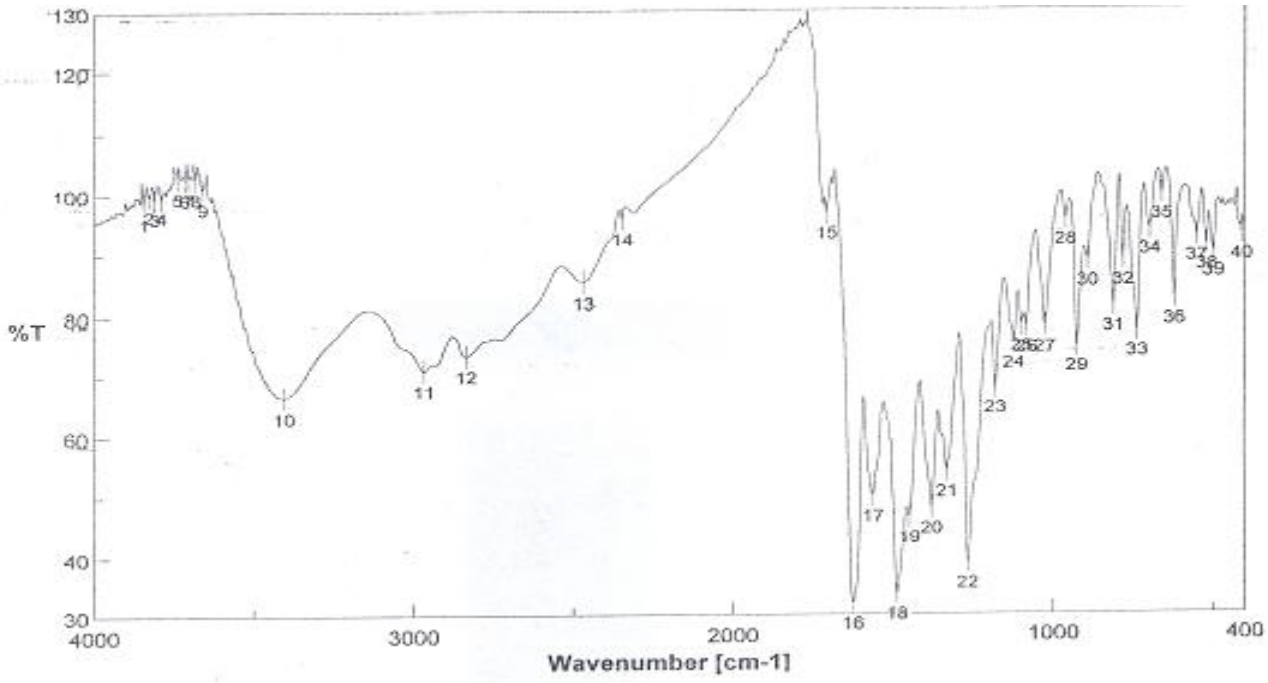

Figure 2: Infrared spectrum of Pd(II) norfloxacin complex

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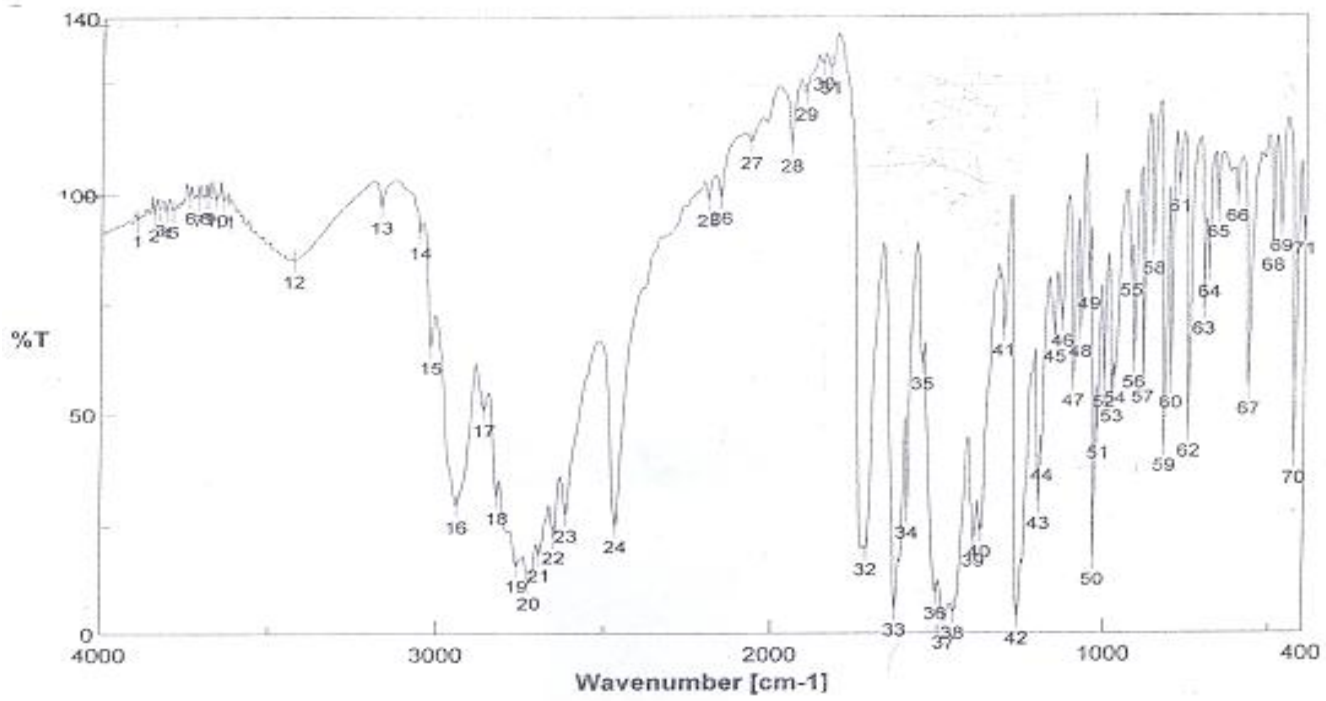

Figure 3: Infrared spectrum of Sb(III) norfloxacin complex

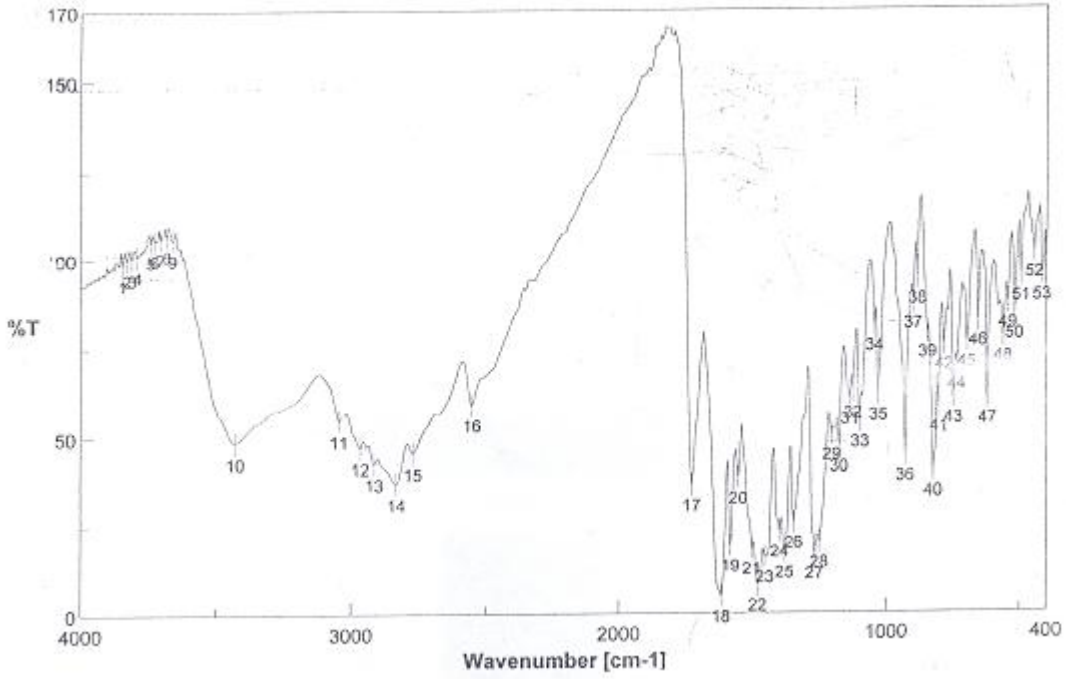

Figure 4: Infrared spectrum of Se(IV) norfloxacin complex

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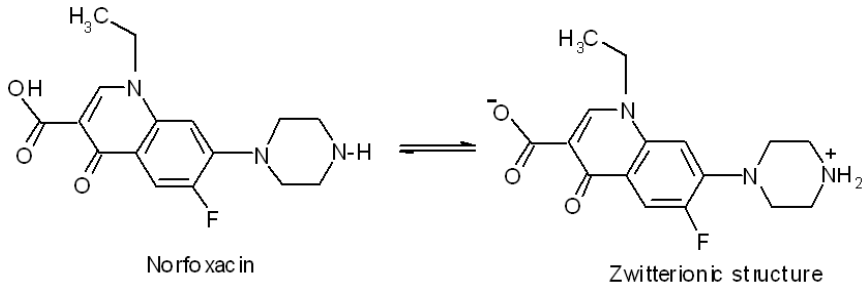
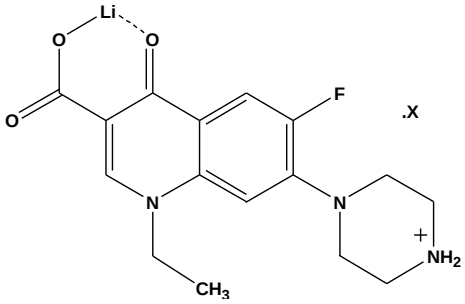
Table 2: Main IR peaks of norfloxacin and its complexes.

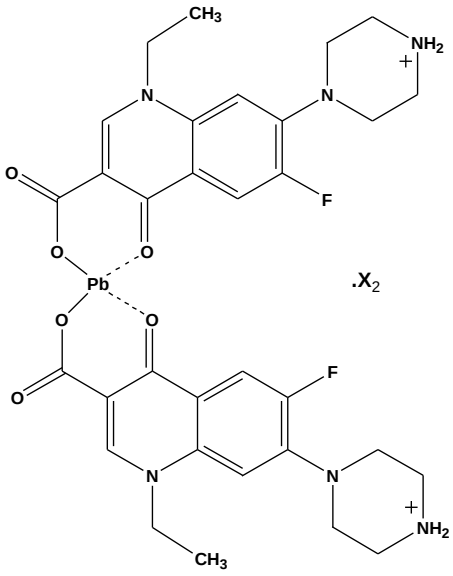
Compound	ν(C=O)	ν(COO) (asym)	ν(COO) (sym.)	ν(C=O) (carbonyl)	ν(M-O)
nor	1727	1590	1396	1716	--
LiCl	--	1580	1383	1628	552, 526, 498, 460
$LiNO_3$	--	1582	1384	1628	571, 521, 478, 460
$LiCH_3COO$	--	1577	1384	1626	552, 526, 498, 459
$PbCl_2$	1710	1565	1383	1626	553, 522, 499
$Pb(NO_3)_2$	1714	1568	1361	1621	550, 526, 505, 468
$SbCl_3$	1716	1594	1377	1627	548, 524, 500, 464
SeO_2	1729	1583	1382	1618	565, 548, 522, 497



Scheme 1: Structure of norfloxacin and its zwitterionic form

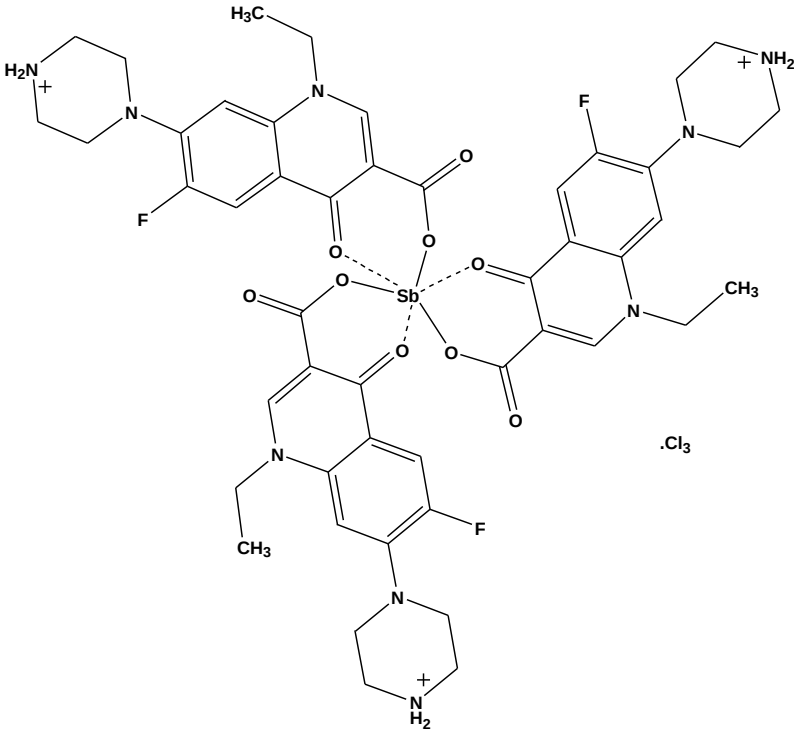


Li(I) norfloxacin complex (X= Cl, NO_3 or OAc)

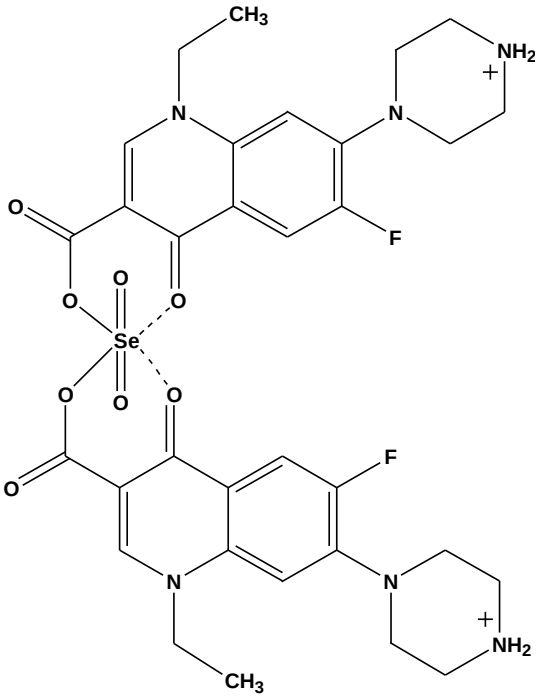


Pd(II) norfloxacin complex (X= Cl_2 or $(NO_3)_2$)

ISSN: 0975-8585



Sb(III) norfloxacin complex



Se(IV) norfloxacin complex

Scheme 2: Structures of Li(I), Pb(II), Sb(III) and Se(IV) norfloxacin complexes

^{1}H-NMR spectra

The ^{1}H-NMR spectra were recorded using a Bruker ARX-300 instrument. Chemical shifts are reported in parts per million downfield from tetramethylsilane. The ^{1}HNMR spectrum of Pb(II) complex on comparing with those ^{1}H-NMR spectrum of the free norfloxacin, indicate that Nor acts as bidentate ligand through the

ring carbonyl oxygen atom and one of the oxygen atoms of the carboxylic group. ^{1}HNMR spectra of lead(II) complex was carried out in DMSO-d_6 as a solvent. The –CH_2– group quartet having a total intensity of two units with the value of δ 4.50, 4.65, 4.70 and 4.80 ppm, while the –CH_3 group (triplet) having a three units of intensity with the value of δ 1.40, 1.45 and 1.48 ppm. This shift is due to the complexation and difference in the configuration of complex than ligand. The data obtained are in agreement with the suggested coordination through the carboxylic group (absent the hydrogen signal of (COOH) in this case) and due to different chemical environments two signals are recorded for the quaternized nitrogen (–$^{+}NH_2$) at δ 2.50 and 2.70 ppm (Scheme 2).

Antimicrobial activity

Antibacterial and antifungal activities of the norfloxacin ligand and its complexes are carried out against the *Escherichia Coli* (Gram -ve), *Bacillus subtilis* (Gram +ve) and antifungal (tricoderma and penicillium activities). The results of the antimicrobial tests are illustrated graphically in Fig. 5. The antimicrobial activity is estimated based on the size of the inhibition zone around the dishes. The norfloxacin complexes are found to have high activity against *E. coli* and *P. rotatum*, whereas the [Li(nor)]Cl and [Pb(nor)$_2$].Cl_2 complexes are more active than the other complexes against *Bacillus subtilis*.

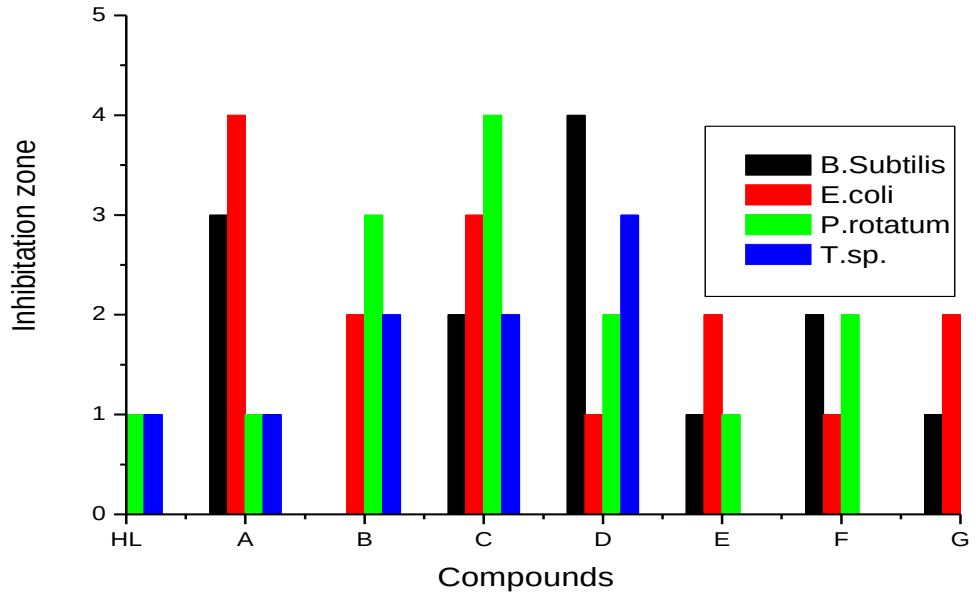


Figure 5: Biological evaluation for HL- norfloxacin and its A- LiCl(I), B- $LiNO_3$(II), C- $LiCH_3COO$(I), D-$PbCl_2$, E- $Pb(NO_3)_2$, F-$SbCl_3$ and G- Se(IV) complexes.

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