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METAL COMPLEXES WITH THE FLUOROQUINOLONE ANTIBACTERIAL AGENT NORFLOXACIN : SYNTHESIS, STRUCTURE AND BIOACTIVITY

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ABSTRACT

Three novel complexes of fluro quinolones antibacterial agent norfloxacin (abbreviated as Nor), $[M(Nor)_2(H_2O)_2]Cl_3.6H_2O$, (M = Fe, Co) and $[Zn(Nor)_2]Cl_2.7H_2O$, have been prepared. The compounds were characterized by IR, UV-Vis, NMR spectra, molar conductivity and elemental analyses. In all of the complexes, the ligand Nor was coordinated through two carboxyl oxygen atoms. Octahedral and tetrahedron geometries have been proposed for Fe (III)-, Co (II)-complexes and Zn (II)-complex, respectively.

Key words : Norfloxacin, Bioactivity, Complex, Zn (II), Fe(III), Co (II).

INTRODUCTION

Fluoroquinolones exhibit potent *in vitro* and *in vivo* antimycobacterial activity[1]. Among the fluoroquinolones, norfloxacin [1-ethyl (-6-fluoro- 1, 4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid] is a synthetic, broad-spectrum antibacterial agent for oral administration. In general, it is active against a wide variety of aerobic gram-negative and gram-positive bacteria. Specifically, the antibacterial spectrum of norfloxacin includes multiantibiotic resistant, gram-negative rods, aminoglycoside-resistant *P. aeruginosa* and beta-lactamase producing organisms. Furthermore, there was little cross-resistance between norfloxacin and agents of other antibiotic classes[2, 3]. The action mechanism of norfloxacin involves inhibition of bacterial DNA gyrase, which is essential for DNA replication[3-5]. The chemical structure of norfloxacin is shown in Fig. 1.

Transition metals are present in very low concentrations *in vivo* and their ligand environment can be considerably altered when a therapeutically effective dose of drug (e. g., an antibacterial agent) is administered. This change in the balance between the metal

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ion and the ligand may have a profound effect upon the activity of the drug against potentially susceptible bacteria.

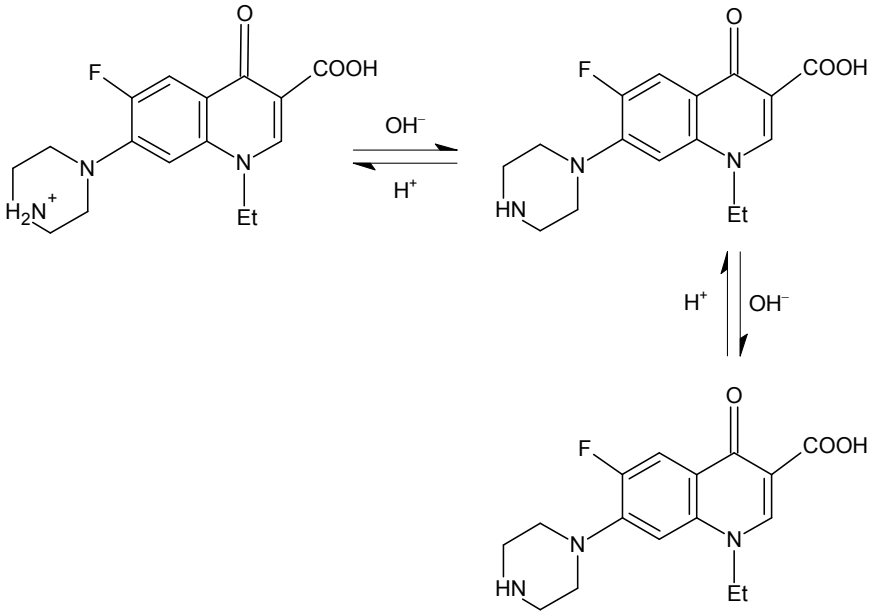

Fig. 1: The zwitterionic structure of norfloxacin molecule

The formation of complexes may increase the bioavailability of the metal ion or the ligand drug, or both. In order to investigate the effect of metal ions upon the antibacterial activity of norfloxacin and probe the mechanism of the drug some novel complexes of norfloxacin with Co^{2+}, Fe^{3+} and Zn^{2+}metal ions have been synthesized. It was found that the solubility of the complexes is better than norfloxacin; both in water and ethanol. On the basis of studies on the IR, UV and ^1H NMR spectra, molar conductivity, melting point measurements, together with elemental analysis, two reasonable structures for these new complexes have been proposed. The antibacterial activity of norfloxacin and its two metal complexes was investigated *in vitro* against several gram-positive (*staphylococcus aureus*) and gram-negative (*E. coli, bacillus dysenteriae*) organisms. It is worth mentioning that the activity of the Zn (II)-Nor complex against a gram-negative organism was remarkably stronger than that of norfloxacin itself.

EXPERIMENTAL

Physical methods

Infrared spectra were recorded by using a Shimadzu IR-435 spectrophotometer.

The compounds were sampled as KCl pellets. ^{1}H NMR spectra were recorded on a Bruker-400 FT NMR spectrometer, 5 mm tubes, TMS as internal standard in D_2O solution. UV-vis spectra were recorded by using a Shimadzu UV-365 recording spectrometer. The metals were titrated with EDTA after destruction by hot concentrated HNO_3. The contents of carbon, nitrogen, hydrogen and oxygen were analyzed by the micro labs in Indian Institute of Technology, Roorkee.

Antibacterial activity tests

In vitro antibacterial activity of the complexes and norfloxacin was tested by using the filter paper scraps diffusion method. The chosen strains include *E. coli*, *Bacillus dysenteriae,* which belong to G (-) strains and *Staphylococcus aureus,* which is a G (+) strain. Small circular scraps of filter paper of diameter 6 mm were made for the purpose of bacteriostatic slices. 10 mg drug (norfloxacin or its metal complex) was dissolved into 10 mL 0.05M, pH 7.4 NaOH--KH_2PO_4 buffer; thus, the concentration was 1 mg/mL. 0.1 mL of the solution was poured into a small bottle that contained ten paper slices and the solution was blotted up by the slices. The bottle was capped by gauze and then disinfected for 20 min under a pressure of 15 lb/in^2 and finally kept in an oven at 80°C for the following tests. A small amount of liquid containing bacterial strain was spread uniformly onto the medium plates with absorbent cotton and the bacteriostatic slices previously prepared were stuck on the medium plates. Every sample was parallel doubled and finally the samples were cultured for 24 hr in order to observe the results.

Starting materials

Norfloxacin (pure powder, 99.9%) was purchased by Sigma Aldrich Company. $ZnCl_2$(AR), $FeCl_3$-$6H_2O$(AR); $CoCl_2.6H_2O$(AR) were purchased from Ranbaxy Fine Chemicals Limited. All other chemicals and reagents used were of analytical grade.

Synthesis of new complexes

Certain amount of norfloxacin (1.0 g, 3.13 m mol) was dissolved into 10 mL 0.1M HCl under slight heating. An appropriate amount of transition metallic chloride salt (corresponding to a molar ratio of 1 : 2 with norfloxacin) was dissolved in 10 mL H_2O; if necessary, the pH was adjusted with 0.1M HCl to avoid the precipitation of metallic hydroxide. By stirring the solution of the metal ions dropped into the solution of norfloxacin, the pH value was increased by adding 0.1M NaOH solution, but it must not lead the precipitation of metallic hydroxide to occur. Generally, a change of color was observed, but precipitation did not occur immediately. With continued stirring, 10 mL methanol was added into the solution and the system was kept at room temperature for

three days. The precipitation of the complex was obtained. The solution was filtered off and the solid was washed repeatedly with ethanol. The compound was dried under vacuum at 50-60°C for 48 hr. The synthesis data are summarized in Table 1.

Table 1.The syntheses of some metal-norfloxacin complexes

Compound	Weight of chloride/g	Weight of norfloxacin/g	pH Value	Color
Fe^{III}-Nor	0.42	1	2.5	red
Co^{II}-Nor	0.37	1	4.5	pale yellow
Zn^{II}-Nor	0.21	1	4.2	white

RESULTS AND DISCUSSION

The interaction of Fe (IlI), Co (II) and Zn (II) with norfloxacin resulted in the formation of the complexes with the formulas $M(Nor)_2Cl_2.8H_2O$ (M = Fe, Co) and $Zn(Nor)_2Cl_2.7H_2O$, corresponding to the analytical data as presented in Table 2.

Table 2 : Analytical data for the complexes

Complex	Analysis Found (Calc.) %					Molar Conductance (Ω^{-1}. cm^2. mol^{-1}) (25°C)
	M	N	C	H	Cl	
$Co(Nor)_2Cl_2.8H_2O$	6.26 (6.47)	9.23 (9.23)	41.73 (42.21)	5.05 (5.53)	- 7.79	122.2
$Fe(Nor)_2Cl_2.8H_2O$	6.37 (6.15)	9.30 (9.26)	41.92 (42.35)	4.92 (5.55)	- 7.81	138.1
$Zn(Nor)_2Cl_2.7H_2O$	7.16 (7.27)	9.22 (9.34)	42.98 (42.75)	4.80 (5.38)	- 7.89	153

The molar conductances in 0.001M methanol are between 100-155 Ω^{-1}. cm^2. mol^{-1} (see Table 2), indicating the 1 : 2 electrolytic nature[6]. The complexes did not melt up to 250°C, while the m. p of norfloxacin is 228°C. All of the complexes were stable in air and

their solubility is much better than norfloxacin itself; both in water and methanol.

Infrared spectral data

The infrared spectral data and their assignments are given in Table 3. The main IR frequencies of metal complexes were compared with that of norfloxacin, (i) There were two very strong absorption peaks in the spectrum of the ligand norfloxacin[7], i. e. 1720 cm^{-1} (COOH) and 1620 cm^{-1} (CO). (ii) Different from the spectrum of the ligand, the band at 1720 cm^{-1} for all the complexes completely vanished, while the strong absorption peak at the position of 1620 cm^{-1} did not change at all. (iii) For the complexes, there appeared two bands in the ranges of 1580-1500 cm^{-1} and 1480-1390 cm^{-1}. Koji Nakashi pointed out[8] that the group --COOH- may emerge as two absorption bands positioned at 1610-1550 cm^{-1} and around 1400 cm^{-1}. Hence, it has been proposed that the ligand norfloxacin interacted with the metal ions through bidentate chelating. This was consistent with the disappearance of the strong absorption at 1720 cm^{-1} in the spectra of complexes. The averaging effect took place between the single and double bonds on the carbonyl group owing to the coordination. (iv) New vibrating absorptions were observed in the range of 550-620 cm^{-1}, which were characterized as the absorption of M--O bonds.

Table 3: Main IR frequencies of norfloxacin and its complexes

Data for Nor itself	norfloxacin and its complexes (cm^{-1}) complex of			Assignments
	Fe (III)	Co (II)	Zn (II)	
1720 vs	--	--	--	νCOOH
1620 vs	1620 vs	1620 vs	1620 vs	ν C = O
1570 sh	1560 sh	1580 sh	1500 m	$\nu_{as}C(=O)O$
1400 m	1390 m	1470 m	1390 m	$\nu_{as}C(=O)O$
580 m	550 m	560 m	620 m	ν M–O

NMR spectra data

For norfloxacin, the assignments of proton magnetic resonance were[9]: δ 1.51 (3H, CH_3), δ 3.61 (8H, piperazine CH_2), δ 4.42 (2H, CH_2), δ 7.02 (IH, 8-H), δ 7.30 (1H, 5-H), δ 8.58 (1H, 2-H) and δ 10.08 (1H, COOH). In all cases for the complexes, except the

vanishing peak at δ 10.08, all the δ values of resonance absorptions changed little, within 0.2 ppm.

UV-Vis spectra data

UV-Vis spectra were studied in aqueous solution with a 1.0 cm quartz cell. The Fe (III)-Nor complex has an absorption maximum at 379 nm. The absorption spectrum of Nor solution (10^{-5}M) was recorded under the same operating conditions against a reagent blank, showing maximum absorption at 278 nm. The maximum absorption peaks of other complexes were all red-shifted 80-100 nm in comparison with Nor.

The structure of the complexes

On the basis of above evidence and analyses, the complexes may be assigned reasonable structures as follows –

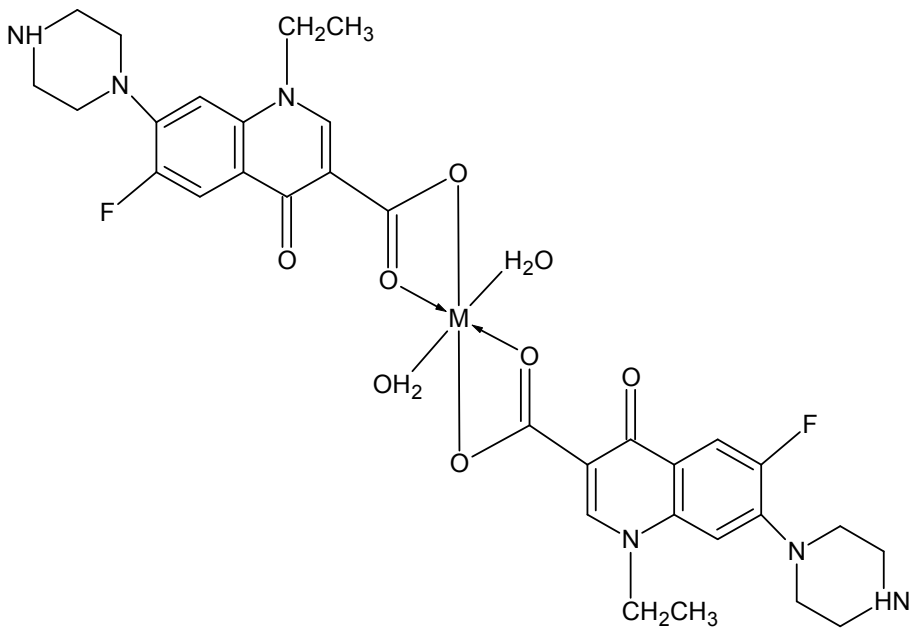

Fig. 2 : The tentative structure of complexes of Fe(III)-Nor and Co(II)-Nor

From the investigation of elementary analyses and molar conductances, the complexes of Fe (III) and Co (II) should have a formula as $[M(Nor)_2]Cl_2.8H_2O$. For Fe (III) and Co (II) ions, the most common coordination number is 6, with an octahedral geometry, and hence, there were two water molecules in inner sphere (the first coordination sphere). The most likely structural scheme of the complexes for Fe (III) and

Co (II) (the charge should be 2+) is shown in Fig. 2. For Zn (II), the coordination number of 4 should be preferable, and hence, there are no water molecules in the inner sphere and the tentative tetrahedral coordination structure can be represented as in Fig. 3.

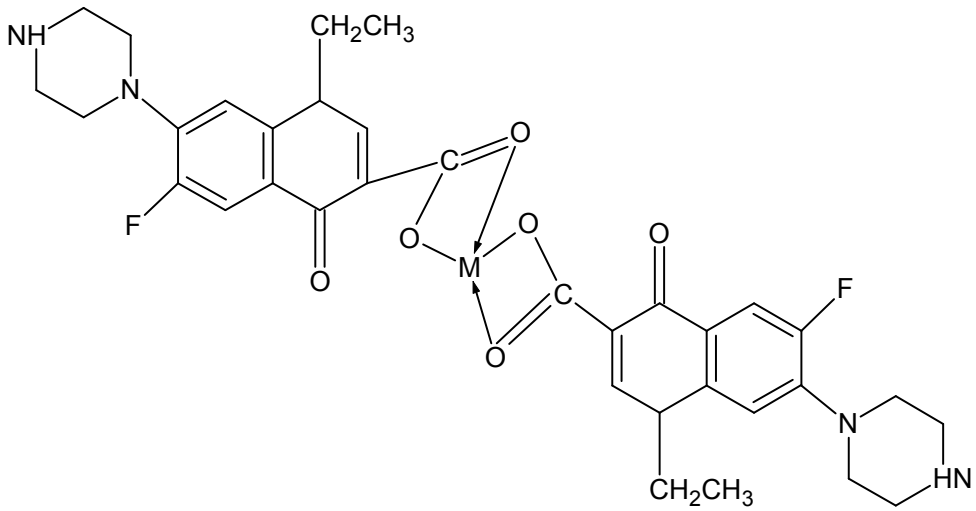

Fig. 3 : The probable structure of the Zn(II)-Nor complex

Biological activity

Shungu et al.[11, 12] suggested that the susceptibility of a certain strain of bacterium towards norfloxacin can be judged by measuring the size of bacteriostatic diameter. For d > 17 mm, it is highly sensitive, while for 13 mm < d < 16 mm, it is intermediately sensitive and d < 12 mm is active.

Table 4. *In vitro* susceptibility test of norfloxacin and its new complexes

Drugs well	Concentration µg/well	Average value of bacteriostatic diameter		
		G (-) *Escherichia Coli*	G (-) *Bacillus Dysenteriae*	G (+) *Staphylococcus Aureus*
Nor	10	39.5	33	32
Fe (III)-Nor	10	36	37	31
Zn (II)-Nor	10	43.5	37	28

For all the results (Table 4), the bacteriostatic diameters were larger than 27 mm, being highly susceptible. The average bacteriostatic diameters of norfloxacin against the chosen strains are: *E. coli*, 39.5 mm; *Bacillus dysenteriae*, 33 mm; and *Staphylococcus aureus*, 32 mm. It was noted that the activity of the zinc complex against G (-) bacteria was considerably stronger than that of norfioxacin itself. As an essential element for life, zinc has multiple biological functions in many physiological processes and its abundance is relatively high. So its complexes formed from coordination with drug molecules may be helpful in the exploration of new chemotherapy agents.

The molecule of norfloxacin is of zwitterionic structure (Fig. 1)[12], having a favorable solubility in acidic or basic solvents, while its solubility in water, methanol, ethanol and chloroform is poor. However, the metal complexes of norfloxacin can fairly dissolve in water, methanol and ethanol. This increase of hydrotropy and liposolubility can enhance the ability of drug molecules in crossing the membrane of a cell and hence, raising the biological utilization ratio and activity of the drug.

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