Asian Journal of Chemistry Vol. 14, Nos. 3–4 (2002), 1287–1292

Effect of Chelation on Antifungal Activity of Norfloxacin

PRAVEEN CHOUBEY and PRAMILA SINGH*
Department of Chemistry, Dr H.S. Gour University, Sagar 470 003, India
E-mail: drpsingh2000@yahoo.com ; praveenchoubey@rediffmail.com

Complexes of norfloxacin, an antibiotic drug with some transition elements, have been prepared and evaluated for their antifungal activity. The complexes of Fe(III), Ni(II) and Cu (II) have been characterized on the basis of analytical data, conductance, magnetic susceptibility measurements and spectral data. When complexes were tested for antifungal activity differences were observed between complex and free ligand (drug). The stoichiometry of the complex has been found to be 1 : 2 for Cu(II) where as Ni(II) and Fe(III) complexes show 1 : 1 stoichiometry with chloride or acetate ion in the coordination sphere. The complexes are of the general formulae $CuL_2.(H_2O)_2$ and MLR (H_2O);. [where R=CH_3COO^- for Ni(II) and Cl^- for Fe(III), L=norfloxacin, M=Cu(II), Ni(II), Fe(III)].

Key Words: Metal complexes, Norfloxacin, Structural, Antifungal studies.

INTRODUCTION

The norfloxacin [3-quinoline carboxylic acid, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)] (Fig. 1) is widely used in the treatment of urinary tract infections caused by *Citrobacter freundii, Enterocloace*, *Kl* pneumoniae and *Streptococci* etc[1]. Chelation shows more potent antifungal effects against some microorganisms than the respective drug[2,3]. The present paper discusses the preparation of complexes of Fe(III), Ni(II) and Cu(II) with norfloxacin and effect of chelation on antifungal activity of the drug.

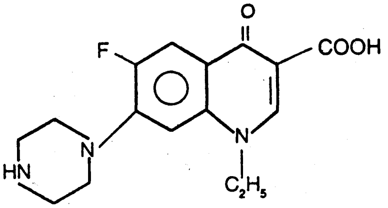


Fig. 1. Norfloxacin

EXPERIMENTAL

All the chemicals used were of AR grade. Doubly distilled water was used throughout. The complexes were isolated from distilled water and concentrated aqueous solution (in the presence of dilute glacial acetic acid) of the drug [L] in slight excess over stoichiometric M : L : : 1 : 1 or 1 : 2 ratio was added slowly with constant stirring to an aqueous solution of metal salt. pH of the solution was so adjusted as to get the indication of complex formation by colour change or by precipitation. All the complexes prepared were obtained in pH range 5–6.5. After adjusting the pH the content was refluxed over steam bath for *ca.* 1 h. The precipitated complexes so obtained were filtered and washed many times with hot distilled water and finally with ether and dried in an oven at about 110°C and stored in a desicccator over anhydrous $CaCl_2$. The purity of the compounds was monitored by TLC using silica gel.

Elemental analyses were carried out on a Heraeus Carlo Erba 1108 analyser at CDRI Lucknow. Metal and chloride contents were determined by standard method[4]. The molecular weights of the complexes were determined by Rast's camphor method. Decomposition temperatures were determined on a Toshniwal CL-0301 apparatus.

The magnetic moments of complexes at room temperature were determined by Gouy's method using $CuSO_4 \cdot 5H_2O$ as standard. The conductivity measurements were carried out in methanol and DMF at a concentration 10^{-3} M using Elico type CM-82T conductivity bridge. The IR spectra [KBr) were recorded on Shimadzu IR-470 infrared spectrophotometer in the range of 4000-400 cm^{-1} and electronic spectra were recorded on Shimudzu 210 A UV/VIS spectrophotometer.

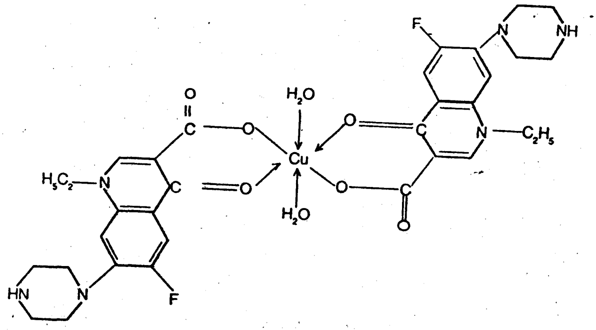
Pregrown cultures of the fungus were inoculated. Sterilized Whatman No. 1 filter paper discs [diameter 5 mm] were thoroughly moistened with the synthesized complex solution to be tested and were placed on seeded agar plates. Petridishes with the fungus were incubated at 20 to 30°C for 24 h. The zone of inhibition was then measured and compared with that of the standard.

RESULTS AND DISCUSSION

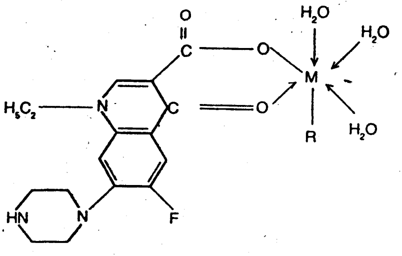
TABLE-1
CHARACTERIZATION DATA OF THE COMPLEXES

Complexes (m.w.) (Colour)	Elemental analysis %, Found (Calcd.)				Molar conductance [Mhos]	μ_{eff} (BM)	m.p./dec. temp. (°C)
	C	H	N	M			
$[Cu(C_{16}H_{17}FN_3O_3)_2(H_2O)_2]$ (736.28) (Light green)	50.19 (52.20)	5.00 (5.16)	10.49 (11.40)	7.71 (8.63)	13.10	1.80	232
$[Ni(C_{16}H_{17}FN_3O_3)(CH_3COO)(H_2O)_3]$ (490.05) (Green)	44.14 (44.11)	5.18 (5.30)	8.43 (8.57)	10.97 (11.97)	10.50	3.20	345
$[Fe(C_{16}H_{17}FN_3O_3)(Cl)(H_2O)_3]$ (463.63) (Brown)	38.61 (41.45)	5.10 (4.96)	8.79 (9.05)	11.69 (12.04)	16.20	5.8	245

The analytical and physical data of the complexes are presented in Table-1. These complexes are microcrystalline, soluble in methanol and DMF. On the basis of elemental analysis Cu(II) complex has 1 : 2 : : M : L stoichiometry with two water molecules and Ni(II) complex has 1 : 1 : : M : L stoichiometry with one acetate ion and three water molecules while Fe(III) complex has 1 : 1 : : M : L stoichiometry with one chloride ion and three water molecules in the coordination sphere. Presence of coordinated acetate ion and water molecules was confirmed by IR data and elemental analysis. The molecular weight (Rast's camphor)[5] data suggest the monomeric nature of the complex. The molar conductances of 10^{-3} M methanol solution of the complexes of Cu(II), Ni(II) and Fe(III) were found to be 13.10, 10.50 and 16.20 $ohm^{-1}cm^{2}mol^{-1}$ respectively. The low value of molar conductance indicates that complexes are non-eletrolytic in nature.



Cu(II) with norfloxacin



M = Ni(II) or Fe(III); R = Cl^- for Fe(III) complex; R = CH_3COO^- for Ni(II) complex

The electronic spectra of copper(II) complex show one broad asymmetric band in the region 13650–12030 cm^{-1}, assignable to $^2E_g \rightarrow {}^2T_g$ transition in distorted octahedral geometry. Magnetic moment value of Cu(II) complex (1.80 BM) shows paramagnetic character[6]. The electronic spectra of Ni(II) complex show bands at 12,550 19,000 and 25,500 cm^{-1}. These bands are assigned to $^3A_{2g} \rightarrow {}^3T_{2g}(V_1)$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)(V_2)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(P)(V_3)$ transitions respectively in conformity with an octahedral geometry[7–9]. Also at room temperature magnetic moment value (3.2 BM) obtained for the Ni(II) complex is indicative of an octahedral geometry for the nickel(II) complex. The Three bands at 13368, 17330 and 23807cm^{-1} are corresponding to $^6A_{1g} \rightarrow {}^4T_{1g}(V_1)$, $^6A_{1g} \rightarrow {}^4T_{2g}(V_2)$ and $^6A_{1g} \rightarrow {}^4T_{1g}(P)(V_3)$, transitions in Fe(III) chelate monomer. Magnetic moment value of Fe(III) complex 5.8 BM shows paramagnetic character and so the octahedral geometry[10].

In the IR spectra of Cu(II), Ni(II) and Fe(III) complexes broad band at *ca.* 3400 cm^{-1} followed by sharp peaks at *ca.* 1600, 830 cm^{-1}assignable to OH strectching bending and rocking vibration respectively indicates the presence of water molecules in the complexes[11]. The band [C=O] which is present in the ligand at 1575 cm^{-1} is shifted by ± 15–20 cm^{-1} in the complexes indicating the coordination through this group. In the far IR region the chelates show new medium intensity bands at *ca.* 482 cm^{-1}, may be due to ν(M—O). [12]In Ni(II) complex additional bands at 1577, 1492, 1448, 1338 and 746 cm^{-1}can be assigned to ν_{asym} (COO), (COO), $\delta(CH_3)$ and δ(COO) respectively indicating the presence of acetate ion in the coordination sphere. Presence of Cl ion in the Fe (III) complex has been confirmed on the basis of elemental analysis.

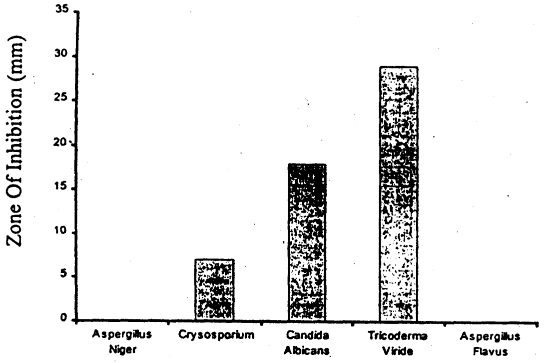
Biological Activity

Antifungal screening of norfloxacin and its metal complexes against the microorganism *Aspergillus niger, Crysosporium, Candida albicans, Tricoderma viride* and *Aspergillus flavus* has been carried out employing filter paper disc diffusion plate method[13].

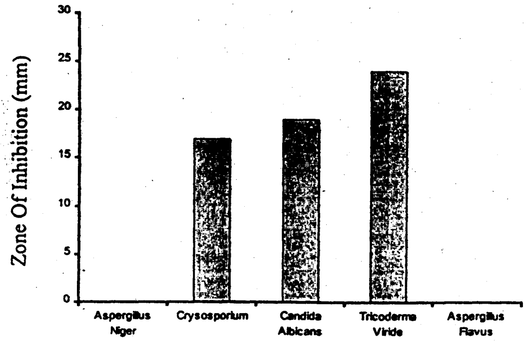
Fig. 2 records the inhibition zone of the complexes at 1 mg/mL concentration against the pathogenic fungus. It has been found that the copper complex showed maximum and remarkable antifungal activity against *Tricoderma viride* and better activity against *Candida albicans* fungus as compared to the parent drug. The Ni(II) and Fe(III) complexes show better acitivity against *Tricoderma viride, Crysosporium* and *Candida albicans* fungus as compared to the parent drug

Higher antifungal activity of certain metal complexes than the pure drug may be due to the fact that complexation with metal imparts some important characteristics to the drug, which are helpful in its fungal activity, *e.g.* low dissociation constant (strong metal bond), special redox potential, electron distribution and solubilities. It also helps in the natural process of bond formation and bond cleavage and the group transfer reactions[14]. As a result, the metal complex has increased duration of action and possesses enhanced blood concentration, which may probably be due to a comparatively faster diffusion of the metal chelate as a whole through the organism due to its more liposoluble nature (more covalent metal-to-ligand bond) on being coordinated with the metal ion forming stable chelates.

Antifungal Effect of Norfloxacin Cu (II) complex



Antifungal Effect of Norfloxacin Ni (II) complex



Antifungal Effect of Norfloxacin Fe (III) complex

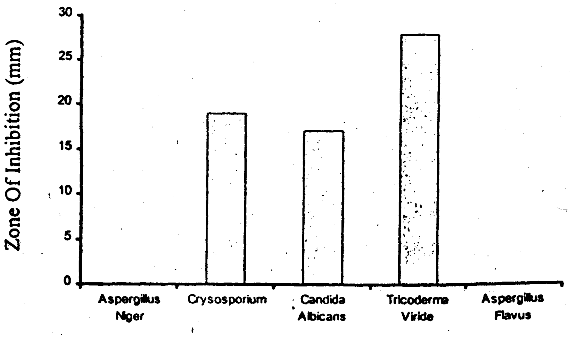


Fig. 2. Antifungal effect of norfloxacin

ACKNOWLEDGMENTS

The authors are thankful to Prof. S.P. Banerjee, Head, Department of Chemistry, Dr. H.S. Gour University, Sagar for providing necessary laboratory facilities, and to CDRI, Lucknow for elemental analysis. We are also thankful to Dr. (Smt.) Archana Mehta Department of Botany, Dr. H.S. Gour University, Sagar for help in to measuring the biological activity.

REFERENCES

1. Remington's Pharmaceutical Sciences, 18th Edn., Mack Pub. Comp., Easton (1990).
2. Goodman and E.S. Gilman, The Pharmacology Basis of Therapeutics, Macmillan, p. 952 (1967),
3. M.B. Chenoweth, *Pharm. Rev.*, **8**, 87 (1956).
4. A.I. Vogel, *A Text Book of Quantitative Inorganic Analysis,* 4th Edn. ELBS and Longmans Green, London, 433, 434, 444 (1978).
5. S. Gladstone, Text Book of Physical Chemistry, 2nd Edn., Mcmillan Co., New York, p. 648 (1946).
6. A.B.P. Lever, Inorganic Electronic Spectroscopy, Elsevier, New York, p. 360 (1968).
7. A.N. Verma, S.B. Ghosle and S.P. Songel, *J. Indian Chem. Soc.*, **69**, 332 (1992).
8. S. Utsuno, *J. Inorg. Nucl. Chem.*, **32**, 163 (1970).
9. R.S. Drago, Physical methods in Inorganic chemistry, Reinhold, New York (1965).
10. B.N. Figgis, Introduction to Ligand Fields Wiley Eastern, New Delhi (1976).
11. I. Camo, *Bull Chem. Soc.*, Japan, **34**, 760 (1961),
12. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds, 3rd* Edn., Wiley Interscience, New York (1978).
13. J.G. Vincent and H.W. Vincent, *Proc. Soc. Exptl. Biol. Med.*, **55**, 162 (1944).
14. A.T. Florence and D. Attwood, Physical Chemical Principles of Pharmacy, Mcmillan, London (1986).

(*Received:* 19 February 2002; *Accepted:* 1 May 2002) AJC-2683