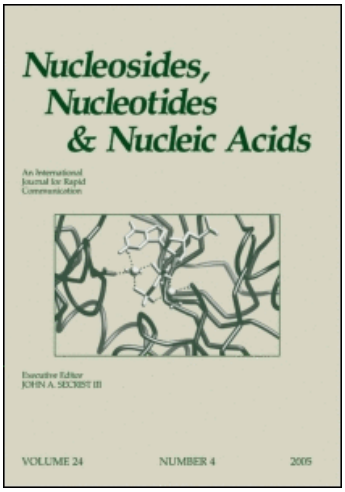

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DNA INTERACTIONS AND PROMOTION IN ANTIBACTERIAL ACTIVITIES OF CIPROFLOXACIN DRUG DUE TO FORMATION OF MIXED-LIGAND COMPLEXES OF OXOVANADIUM(IV)

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□ *Mixed-ligand complexes of oxovanadium(IV) of the type [VOAL]·2H_2O [where A = ciprofloxacin and L = uninegative bidentate ligands] have been synthesized and characterized using infrared spectra, electronic spectra, magnetic measurements, elemental analyses, thermal investigation, and mass spectroscopy. Here, we tried to increase an antibacterial activity of ciprofloxacin drug due to formation of mixed-ligand complexes. The complexes were found to be more potent compare to some standard drugs, ligands and metal salt against selective gram(+ve) and gram(−ve) organisms. Binding of the complexes with DNA have been investigated by spectroscopic absorption titration and viscometric techniques. The mixed-ligand complexes show good binding ability. The cleavage efficacy has been determined using gel electrophoresis method and complexes were found to be more active compared to parental ligands and metal salt.*

Keywords Oxovanadium(IV); MIC; DNA binding constants(K_b); absorption titration; gel electrophoresis and viscometric techniques

INTRODUCTION

Numerous biological experiments performed so far suggest that DNA is the primary intercellular target of anticancer drugs because the interaction between small molecules and DNA can cause damage in the DNA of cancerous cell, which inhibits the cell division and results in the death of cell.[1–2] The interaction of transition metal complexes containing multidentate ligands with DNA has recently gained much attention followed by an important biological and medical roles played by potential metallointercalators.[3–4] It is necessary to understand the binding properties for development of new DNA targeting drugs. Basically, metal complexes interact with double helix

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DNA in either non-covalent or covalent way. The former way includes three binding modes namely intercalation, grooves binding and external static electronic effect. Among these, intercalation is one of the most important DNA binding modes.[5] The biological significance of vanadium was recognized nearly 100 years ago. It was found that the complex of vanadium and ciprofloxacin is promising with respect to its insulin mimetic behavior and concomitant low toxicity in the physiological concentration range.[6] The tests on the potential antibacterial properties have been performed in view of the fact that increasing incidence of antibiotic resistance has brought a new sense of urgency to the discovery and development of new antibacterial drugs.[7] In order to continue research on DNA- binding of quinolone and its transition metal ion complexes,[8–12] in this article, we have prepared the oxovanadium(IV) complexes with ciprofloxacin and uninegative bidentate ligand such as 1-(2-hydroxyphenyl)ethanone (L^1), salicylaldehyde (L^2), 2-hydroxy-1-naphthaldehyde (L^3), 2-hydroxy-5-methyl-propiophenone (L^4), 5-bromosalicylaldehyde (L^5), 3,5-dibromosalicylaldehyde (L^6). The DNA-binding and cleavage properties of the complexes have been investigated by ultraviolet spectroscopy, viscosity measurements.

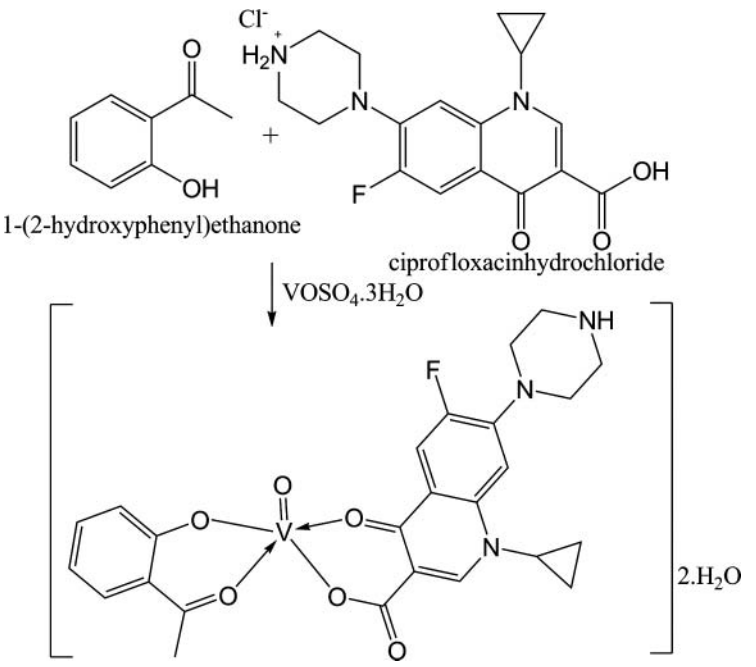
EXPERIMENTAL

Materials

All the chemicals used were of analytical grade. Vanadylsulphate, 1-(2-hydroxyphenyl)ethanone (L^1), salicylaldehyde (L^2), 2-hydroxy-1-naphthaldehyde (L^3), 2-hydroxy-5-methyl-propiophenone (L^4), 5-bromosalicylaldehyde (L^5), 3,5-dibromosalicylaldehyde (L^6) were purchased from, E. Merck Ltd. (Mumbai, India). Ciprofloxacin hydrochloride was purchased from Bayer AG (Wyppertal, Germany). Luria broth, ethidium bromide, sucrose, tris(hydroxymethyl)methylamine, and agar-agar were purchased from Hi-Media Laboratories Pvt. Ltd., India. Agarose was purchased from Sisco Research Laboratories, India. Bromophenol blue, acetic acid, and EDTA were purchased from SD Fine Chemicals, India. The organic solvents were purified by standard methods.[13]

Synthesis of the Mixed-Ligand Complexes

An ethanolic solution (50 mL) of $VOSO_4.3H_2O$ (0.6327 g, 2.5 mM) was added to an ethanolic solution of (50 mL) 1-(2-hydroxyphenyl)ethanone (L^1) (0.384 ml, 2.5 mM), followed by addition of formerly primed solution of H_2CipCl (0.917 g, 2.5 mM) in water and pH of the reaction mixture was adjusted to 6.0∼7.0 pH with dilute NaOH solution. The resulting green solution was refluxed with stirring for 7 hours, and then heated in a steam bath to evaporate up to half volume. The reaction mixture was kept for



SCHEME 1 Probable structure of the complex I.

overnight at room temperature. A fine green colored product was obtained. The obtained product was washed with ether and dried over vacuum desiccators. The probable structure of the complex I is shown in Scheme 1. The compounds II-VI were prepared according to the same method and their physicochemical parameters are summarized in Table 1.

Physical Measurements

Perkin Elmer elemental analyzer (240; USA) was used to analyze carbon, hydrogen, and nitrogen. Thermogravimetric analysis and differential

TABLE 1 Experimental and physical parameters of the complexes

Complexes empirical formula	Elemental analysis% found (required)				m.p. °C	% Yield	Formula weight (gm/mol)
	C	H	N	M			
$C_{25}H_{28}FN_3O_8V$ (I)	52.82(52.84)	4.96(4.94)	7.39(7.38)	8.96(8.95)	>360	61.2	568.45
$C_{24}H_{26}FN_3O_8V$ (II)	51.99(51.97)	4.73(4.70)	7.58(7.60)	9.19(9.16)	>360	65.5	554.42
$C_{28}H_{28}FN_3O_8V$ (III)	55.63(55.61)	4.67(4.64)	6.95(6.93)	8.43(8.46)	>360	59.5	604.48
$C_{27}H_{32}FN_3O_8V$ (IV)	57.47(57.36)	5.41(5.44)	7.04(7.05)	8.54(8.59)	>360	67.8	596.50
$C_{24}H_{25}BrFN_3O_8V$ (V)	45.23(45.52)	4.02(3.98)	6.54(6.63)	8.03(8.04)	>360	63.4	633.31
$C_{24}H_{24}Br_2FN_3O_8V$ (VI)	40.62(40.47)	3.38(3.40)	5.84(5.90)	7.25(7.15)	>360	58.0	712.21

scanning calorimetric study were performed with a model 5000/2960 SDTA, TA instrument. Infrared spectra were recorded on an FTIR (Shimadzu, Japan) spectrophotometer as KBr pellets in the range of 4000 to 400 cm^{-1}. The electronic spectra of the complexes were recorded in the range of 800 to 200 nm on UV-160A UV-vis spectrophotometer (Shimadzu). The magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20°C; Citizen Balance, USA). The diamagnetic correction was made using Pascal's constant. The FAB mass spectra were recorded on a Jeol SX 120/Da-600 mass spectrometer/data system using Argon/Xenon(6 Kv, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature.

Antibacterial Studies

All the bacterial strains were inoculated in 100 mL Luria broth containing Erlenmeyer flasks with shaking at optimum temperatures until respective log-growth phase was achieved. The compounds were dissolved in DMSO and then diluted using Luria broth. Two-fold serial concentrations of the compounds were employed to determine the (MIC) ranging from 100 to 0.1 μM. Test cultures were incubated at 37°C (24 hours). The lowest concentrations of antimicrobial agents that resulted in complete inhibition of microorganisms were determined as (MIC) μM for respective bacterial strains. In each case triplicate tests were performed and the average was taken as the final reading.[14]

DNA Binding and Cleavage Experiments

UV visible absorption spectra of the complexes were recorded on a Shimadzu model UV spectrophotometer at room temperature. A solution of sperm herring DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm about 1.8–1.9, indicating that the DNA was sufficiently free from protein.[15] The concentration of sperm herring DNA was determined spectrophotometrically using the molar extinction coefficient at 6,000 M^{-1} cm^{-1} at 260 nm.[16] Stock solutions were stored at 4°C and used within 48 hours of preparation. The complex and DNA solutions were allowed to incubate for 10 minutes before the absorption spectra were recorded. Absorption titration was carried out by varying the DNA concentration (0–100 μM) and maintaining constant concentration of the complex (4 μM). The electronic absorption spectra of complexes in phosphate buffer pH 7.2 were performed by using a fixed complex concentration to which increasing amounts of DNA stock solution were added. To enable quantitative comparison of the DNA binding affinities the intrinsic binding constant K_b of the complexes for binding with sperm herring DNA were obtained by using equation given

below.[17–19]

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)},$$

where [DNA] is the concentration of DNA in terms of nucleotide phosphate, [NP] the apparent absorption coefficient ε_f, ε_a and ε_b correspond to the extinction coefficient of the free complex, for each addition of DNA to the complex and the complex in the fully bound form respectively and K_b is the ratio of the slope to the y intercept. The binding constant, K_b, for the complex has been determined from the plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus $[DNA]$.

Viscosity measurements were carried out using an Ubbelohde viscometer maintained at a constant temperature 27.0 ± 0.1°C in a thermostatic bath. Flow time was measured with a digital stopwatch in triplicate, and an average flow time was considered. Data are presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio,[20–21] where η_0 and η are the viscosity of DNA in the absence and presence of complex, respectively.

The plasmid DNA pUC19 (4,363 base pairs in length, density of supercoiling, r = −0.065), was prepared by transformation of pUC19 into safe competent cells (*Escherichia coli* strain), amplification of a clone as outlined.[22] After concentration by ethanol precipitation, DNA was stored in TE buffer (pH 8.0) at −20°C. The relative amount of the supercoiled (SC) form was checked by gel electrophoresis on agarose. The preparations contained about 100% of the SC form and 0% of the open circular (OC) form. Electrophoresis was carried out in a submarine mini-gel electrophoresis unit. Supercoiled pUC19 DNA (200 ng) in Tris–HCl buffer (50 mM) containing 50 mM NaCl (pH 7.4) was treated with nickel complexes to yield a total volume of 10 μl and then incubated in dark for 1.5 hours at 37°C. The reaction was quenched by the addition of 3 μl loading buffer, and then the resulting solutions were loaded on a 1.5% agarose gel. Electrophoresis was carried out at 50 V for 2 hours in TAE buffer (mM Tris-acetate and 2 mM EDTA (pH 8.0)). DNA bands were visualized under UV light and photographed. The quantification of each form of DNA was made by densitometric analysis of ethidium bromide containing agarose gel, by using the volume quantization AlphaDigiDoc RT. Version V.4.1.0 PC-Image software (USA). Note that small differences in staining make exact quantitative gel-to-gel comparisons difficult. Densitometry is uncorrected for differential uptake of EtBr by SC and non-SC DNA. A previous study with pUC19 plasmid under similar conditions showed this factor was small.[11]

RESULTS AND DISCUSSION

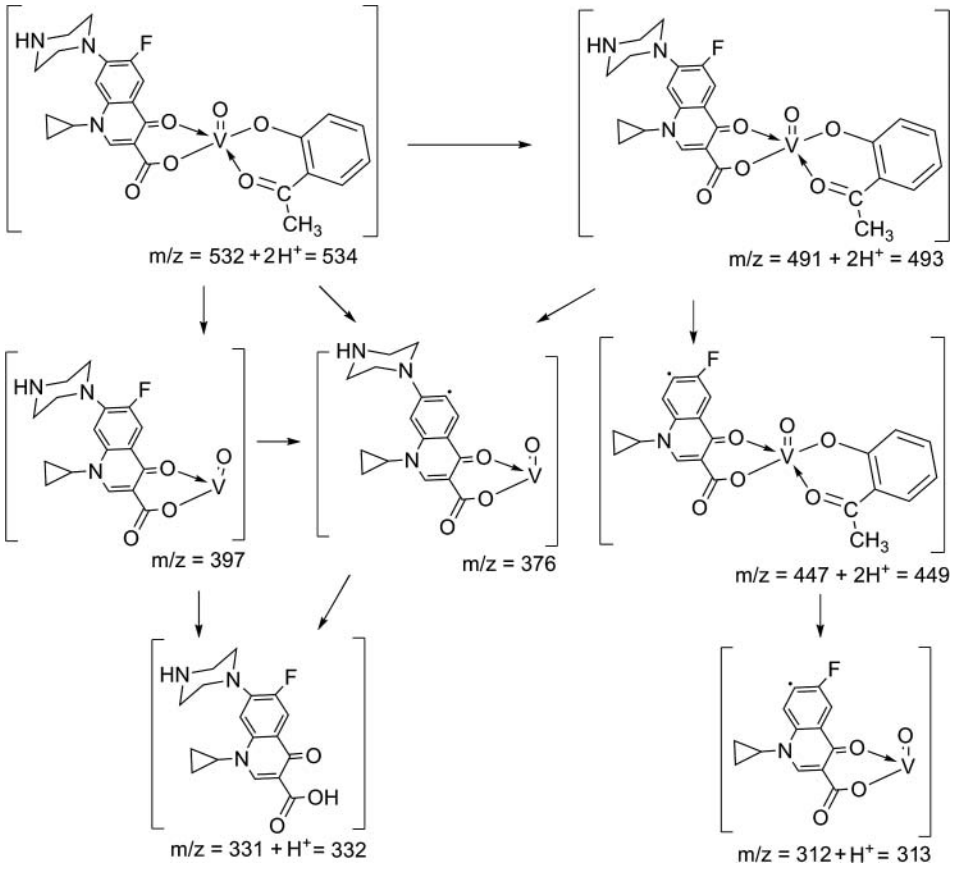
All the synthesized complexes are stable to air for extended period of time and soluble in DMSO, slightly soluble in ethanol and water; insoluble

in benzene, acetone, acetonitrile, and diethyl ether. Elemental analyses of the complexes are in good agreement with theoretical expectation. They possess high melting points indicating that the complexes are stable in air.

The ν(C=O) stretching vibration band appears at 1708 cm^{-1} in the spectra of ciprofloxacin, while in mixed-ligand complexes this band shifted toward lower energy at 1618–1690 cm^{-1}; suggests that coordination occurs through pyridone oxygen atom.[23–24] The absorption bands observed at 1624 and 1340 cm^{-1} in ciprofloxacin are assigned to be $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in mixed-ligand complexes these bands observed at 1586–1599 and 1370–1373 cm^{-1}. The frequency separation ($\Delta\nu = \nu(COO)_{asy}—\nu(COO)_{sym}$) in investigated mixed-ligand complexes is greater than 200 cm^{-1}, suggests that the carboxylato group posses unidentate nature.[25] The sharp band in ciprofloxacin at 3520 cm^{-1} is due to hydrogen bonding;[11] which is attributed to ionic resonance structure and peak observed because of stretching vibration of free hydroxyl group. This band absolutely vanished in the spectra of mixed-ligand complexes indicates deprotonation of carboxylic proton. Of particular interest are the spectroscopic features of the conformers in the 1100–850 cm^{-1} region, which after screening the bands due to ligand internal stretching, reveal interesting differences in their metal-terminal oxygen (V=O_t) vibrational modes. The metal-terminal oxygen (V=O_t) band observed at ~1000 cm^{-1} region suggesting the anti conformers and vanadium has a distorted square pyramidal structure.[26] The complexes have one unpaired electron, characteristic of vanadyl unit, and a fairly high V=O stretching frequency in the infra red spectrum about 1000 cm^{-1}, suggesting that there is no ligand (or a weakly bound solvent) in the sixth position.[12] Some prominent infrared (IR) band frequencies of the compounds are provided in Table 2.

The UV–vis spectra of the complexes have been recorded using UV-160A UV-Vis. spectrophotometer (Shimadzu). The four absorption bands are observed in mixed-ligand complexes at ~800 nm for $d_{xy} \rightarrow d_{xz}$ (band I), ~580 nm for $d_{xy} \rightarrow d_{yz}$ (band II), at ~530 nm for $d_{xy} \rightarrow d_{x^2-y^2}$ (band III), and ~405 nm for $d_{xy} \rightarrow d_{z^2}$ (band IV). An examination of Table 3 substantiates that the parameter Δ (λ_1-λ_2), related to the splitting of the d_{yz} and d_{xz} levels, can be used for establishing the geometrical distortion. In particular, Δ (λ_1–λ_2), increases with increasing distortion in the following order of complexes: III (165nm) < V (171 nm) < IV (194nm) < II (203 nm) < VI (204 nm) < I (223 nm). The mixed-ligand complexes exhibit magnetic moment of 1.69–1.73 B.M. This value is close to the spin-only value expected for s = 1/2 system (1.73 B.M.) and may be indicative of distorted square pyramidal geometry around the VO(IV) ion.

The mass spectra of the complex (I) ($C_{25}H_{26}FN_3O_6V$) revels that peak at m/z = 534 stands for the molecular ion peak of complex (without water of crystallization) where as peaks observed at 493, 449, 397, 376, 332, 313 m/z values correspond to the fragments as shown in Figure 1 and Scheme 2.



SCHEME 2 Probable mass fragmentation pattern for complex I.

The thermogravimetric analyses for the mixed-ligand complexes were carried out within a temperature range from 20 to 800°C in N_2 atmosphere at a rate of 10°C per minute in order to establish their compositional differences as well as to ascertain the nature of associated water molecules.[12] The determined temperature ranges and corresponding percent mass loss accompanying the changes in the mixed-ligand complexes on heating revealed the following things. The TG curves of mixed-ligand complexes show three-decomposition steps respectively. It has been observed that all the

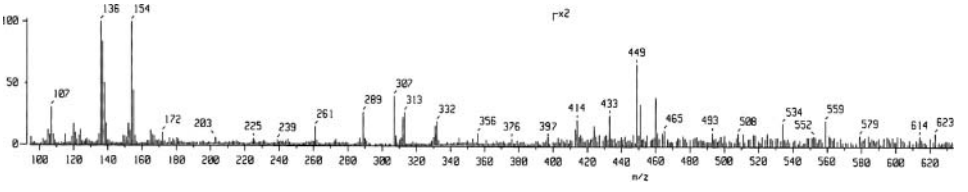


FIGURE 1 Mass spectra of the complex ($C_{25}H_{26}FN_3O_6V$) I.

TABLE 2 Infrared spectral data of the complexes

Complexes	ν(C=O) pyridone	$\nu(COO)_{sy}$	$\nu(COO)_{asy}$	$\Delta\nu$	$\nu(M-O)_{ph}$	$\nu(M-O)_{Carbo}$	$\nu(M-O)_{Keto/aldehyde}$	ν(C−F)	ν(C−N)	ν(V=O)
I	1618	1374	1587	213	470	426	513	1276	1356	1001
II	1618	1374	1587	213	461	426	513	1276	1356	1001
III	1620	1371	1586	215	471	427	514	1275	1357	1020
IV	1619	1371	1587	216	475	428	513	1277	1356	1021
V	1678	1373	1589	216	470	427	535	1278	1354	1020
VI	1680	1372	1585	213	472	426	532	1274	1357	1020

TABLE 3 Electronic absorption parameters of VO(IV) complexes

Complexes	λ_4	λ_3	λ_2	λ_1	Δ (λ_1–λ_2)
I	396	544	606	829	223
II	401	548	598	801	203
III	408	530	577	835	165
IV	410	538	588	829	194
V	398	548	598	771	171
VI	401	544	576	780	204

mixed-ligand complexes show a loss in weight corresponding to two water molecules in the range of 50 to 130°C which indicating that these water molecules are water of crystallization. In second step weight loss during 130–420°C is corresponding to liberation of ligands and leaving behind the oxide of metal in the temperature range 420 to 710°C. Thermal analysis data for complexes are given in the Table 4.

A stock solution of 3000 μM was prepared by dissolving each compound in DMSO solution. The antimicrobial activity test was screened by minimal inhibitory concentration (MIC). MIC was determined with the help of progressive double dilution method[27–28] in liquid media containing compound with a varying range of 0.1 to 3000.0 μM. The efficiencies of the ciprofloxacin, ligands, metal salt, and their mixed-ligand complexes were screened against three gram(−ve), that is *E. coli, S. merscences* and *P. aeruginosa*, and two gram(+ve), that is, *S. aureus* and *B. subtilis* microorganisms. The results are presented in Table 5. In general, the antimicrobial activity

TABLE 4 Thermal analysis data of the complexes

Complexes	TG range/°C	Assignment
I	50–120	Loss of two lattice water molecules
	150–410	Removal of the ligands
	410–710	Leaving V_2O_5 as residue
II	50–130	Loss of two lattice water molecules
	160–410	Removal of the ligands
	450–700	Leaving V_2O_5 as residue
III	50–120	Loss of two lattice water molecules
	150–420	Removal of the ligands
	420–680	Leaving V_2O_5 as residue
IV	50–130	Loss of two lattice water molecules
	150–400	Removal of the ligands
	410–700	Leaving V_2O_5 as residue
V	50–120	Loss of two lattice water molecules
	130–390	Removal of the ligands
	410–650	Leaving V_2O_5 as residue
VI	50–130	Loss of two lattice water molecules
	150–430	Removal of the ligands
	410–690	Leaving V_2O_5 as residue

TABLE 5 MIC data of the compounds (μM)

Compounds	Gram(+ve)		Gram(−ve)		
	S. aureus	*B. subtilis*	*S. merscences*	*P. aeruginosa*	*E. coli*
$VOSO_4 \cdot 3H_2O$	2568.00	2568.00	2568.00	1580.00	1185.00
Ciprofloxacin (cip.)	1.63	1.08	1.63	1.36	1.36
Gatifloxacin	5.06	3.99	2.93	1.01	2.93
Norfloxacin	2.50	2.50	4.07	3.75	2.81
Enrofloxacin	1.94	3.89	1.66	1.39	1.39
Pefloxacin	2.09	2.39	5.09	5.69	2.69
Levofloxacin	1.66	2.21	1.66	1.66	0.96
L^1	1637.73	1310.19	1719.62	1146.41	2292.83
L^2	1028.28	1028.28	1175.17	881.38	1542.42
L^3	929.26	464.63	1045.42	348.47	1045.42
L^4	609.01	487.21	730.82	487.21	974.42
L^5	198.99	99.49	298.48	149.24	149.24
L^6	71.45	28.58	142.90	35.73	28.58
I	1.76	0.88	1.76	0.88	1.76
II	2.71	0.54	0.90	1.80	2.71
III	1.65	0.83	1.65	0.83	0.83
IV	2.51	3.35	3.35	3.35	3.35
V	3.16	1.58	1.58	3.16	1.58
VI	1.40	1.40	0.70	0.70	0.70

of all the complexes against the five microorganisms is much higher than metal salt and ligands (L^1–L^6), while in competition with the ciprofloxacin. The results of our study indicate that the compounds VI show the best activity against all organisms excluding *B. subtilis* in which it is comparable with ciprofloxacin, while in case of *B. subtilis* compound I, II, III comprise good activity compared to standard drugs. It is also notable that compound I, II, III, VI can be potent antibacterial for both gram(−ve) as well as gram(+ve) organisms. As the number of aromatic ring increases, minimum inhibition activity of complex III increases. 5-Bromosalicylaldehyde (L^5) and 3,5-dibromosalicylaldehyde (L^6) show better minimum inhibition activity against all organisms, as it contains one and two -bromo groups, respectively. Similarly complexes V and VI show better minimum inhibition activity against all organisms. But complex VI shows better minimum inhibition activity than complex V as it contains dibromo derivative.

This enhancement in the activity can be explained on the basis of chelation theory and/or may be due to Overtone's concept.[29–30] Chelation reduces the polarity of the metal ion considerably, mainly because of the partial sharing of its positive charge with donor groups and possible π-electron delocalization on the whole chelate ring. The lipids and polysaccharides are some important constituents of cell walls and membranes, which cell wall also contains many aminophosphates, and carbonyl and cysteinyl ligands,

which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Chelation can considerably reduce the polarity of the metal ion, which in turn increases the lipophilic character of the chelate. Thus, interaction between metal ion and the lipid is favoured. This may lead to the breakdown of the permeability barrier of the cell, resulting in interference with the normal cell processes. If the geometry and charge distribution around the molecule are incompatible with the geometry and charge distribution around the pores of the bacterial cell wall, penetration through the wall by the toxic agent cannot take place and this will prevent the toxic reaction within the pores. In addition to these, some important factors that contribute to the activity are the nature of the metal ion, the nature of the ligand, coordinating sites and geometry of the complex, concentration, hydrophilicity, lipophilicity and the presence of co-ligands. Certainly, steric and pharmokinetic factors also play a decisive role in deciding the potency of an antimicrobial agent. The presence of lipophilic and polar substituents is expected to enhance antibacterial activity. Heterocyclic ligands with multifunctionality have a greater chance of interaction either with nucleoside bases (even after complexation with metal ion) or with biologically essential metal ions present in the biosystem, and can be promising candidates as bactericides since they always look to enact especially with some enzymatic functional groups, to achieve a higher coordination number. Thus, the antibacterial property of metal complexes cannot be ascribed to chelation alone but it is an intricate blend of all the above contributions.

DNA Interaction to the Complexes

DNA can provide three distinctive binding sites for the quinolone complexes; namely, groove binding, binding to phosphate group and intercalation. This behavior is of great importance with regard to the relevant biological role of fluoroquinolone antibiotics in the human body.[31]

Absorption Spectral Studies

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques. Complex binding with DNA through intercalation usually results in hypsochromism and bathochromism, due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The absorption spectra of the complex in the absence and presence of DNA are illustrated in Figure 2. Change in absorbance at peak maximum shows moderate hypsochromism shift (~2 nm). In the plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA], the binding constant K_b is given by the ratio of the slope to the intercept. The binding constant for VO(IV) complexes varies in the range of 7.0×10^3 to $6.0 \times 10^4\ M^{-1}$, all data are given in the Table 6. The DNA-binding constant

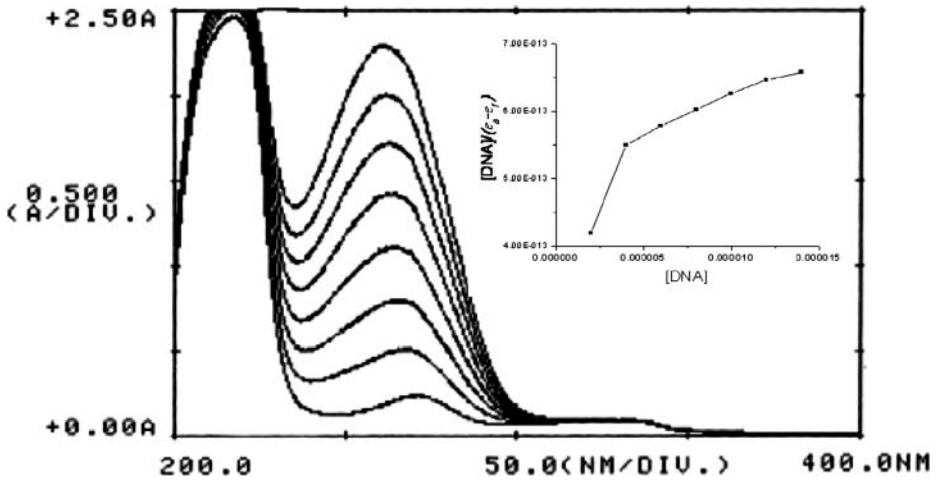


FIGURE 2 Absorption titration spectra of ($C_{25}H_{26}FN_3O_6V$) I.

of the title complexes are comparable to those of some DNA intercalative Ru(II) complexes 1.1–4.8 $\times$ 10^4 M^{-1}.[32–33]

Viscosity Measurements

To further clarify the interactions between the complexes and DNA, viscosity measurements were carried out. Optical photophysical probes provide necessary, but not sufficient clues to support a binding model. Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data.[32–34] A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial intercalation ligand could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity. The effects of the complexes on the viscosity of DNA at 27.0 ($\pm$0.1)°C are shown in Figure 3. The viscosities of the DNA increase with increasing amounts of the complexes. Such behavior is

TABLE 6 The binding constants (K_b) of VO(IV) complexes with DNA in Phosphate buffer pH 7.2

Complexes	K_b (M^{-1})
I	6.0×10^4
II	1.0×10^4
III	7.0×10^3
IV	2.0×10^4
V	1.0×10^4
VI	8.0×10^3

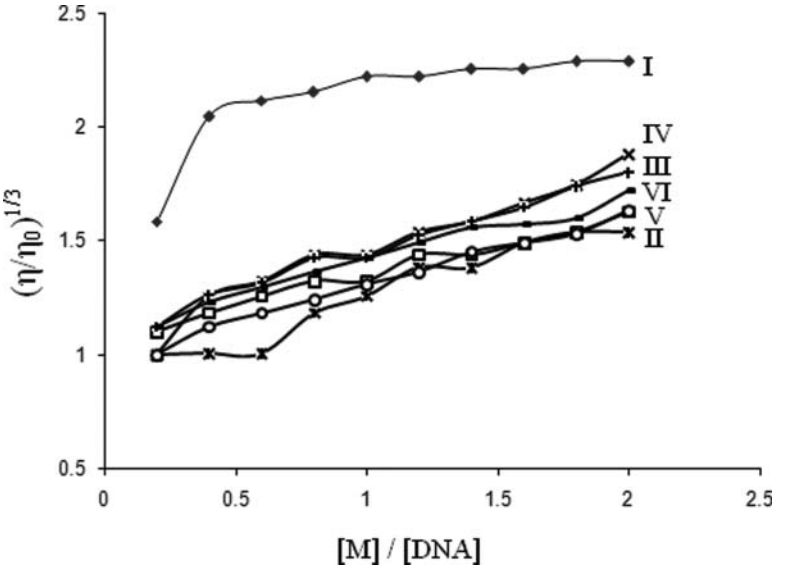


FIGURE 3 The effects of the complexes on the viscosity of DNA.

consistent with other intercalators (i.e., EB). It clearly shows that complexes can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the viscosity of DNA.[35] The results obtained from viscosity studies also validate those obtained from the spectroscopic studies.

DNA Nuclease Activity of Complexes

The ability of the $V^{(IV)}O$ complexes in effecting DNA cleavage has been studied by gel electrophoresis using pUC19 DNA. Figure 4 shows the gel electrophoretic separations of plasmid pUC19 DNA after 1 hour incubation in the presence of $V^{(IV)}O$ complexes (50 μM) and Figure 5 shows the relative percentage of intensity of three forms produced due to the reaction of pUC19 DNA and compounds.

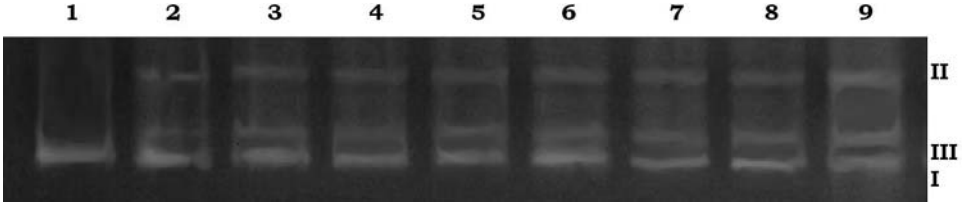


FIGURE 4 Agarose gel electrophoresis of pUC19 DNA with compounds (A) lane 1: pUC19 (control); lane 2: pUC19 + H_2Cip.Cl; lane 3: pUC19 + $VOSO_4$; lane 4: pUC19 +I; lane 5: pUC19 +II; lane 6: pUC19 +III; lane 7: pUC19 +IV; lane 8: pUC19 +V; lane 9: pUC19 +VI.

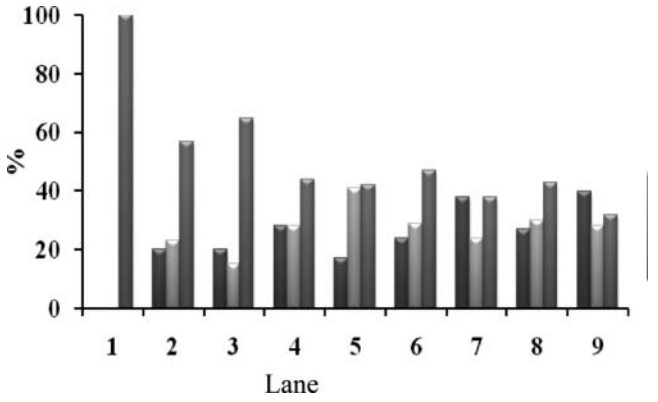


FIGURE 5 The relative percent intensity of three forms of pUC19 DNA in presence of metal complexes (A) lane 1: pUC19 (control); lane 2: pUC19 + H_2Cip.Cl; lane 3: pUC19 + $VOSO_4$; lane 4: pUC19 +I; lane 5: pUC19 +II; lane 6: pUC19 +III; lane 7: pUC19 +IV; lane 8: pUC19 +V; lane 9: pUC19 +VI.

Interaction of pUC19 DNA to the complexes is typical example of intercalative mode.[12] From the experiment, it was observed that the complexes make conformational changes on plasmid DNA by making single strand nicking (NC) or by unwinding the SC plamid DNA to open circular (OC) forms. The electrophoresis experiment showed that the interaction of the complexes with DNA induce strand breakages. In addition, it was also observed that change in intrinsic viscosity provide absolute proof of intercalative binding.

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