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Synthesis, spectroscopy, thermal and biological aspect of novel six-coordinated dimeric iron(III) mixed-ligand complexes

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The mixed-ligand complexes of iron(III) with 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid and various neutral bidentate Schiff base ligands were prepared. The structure of mixed-ligand complexes was investigated using spectral, physicochemical and elemental analyses. Biocidal activity was determined using agar plate technique against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhi, Escherichia coli* and *Serratia marcescens*. The result showed a significant increase in a biocidal activity compared with parent ligands, metal salts and standard drugs (ofloxacin, levofloxacin). DNA binding and cleavage studies were carried out using absorption titration and gel electrophoresis techniques, respectively. The binding constant of Fe(III) complexes was obtained in the range 2.5–4.0 × 10^4 M^{-1}. The DNA binding and cleavage efficacy were raised in mixed-ligand complexes as compared with parental ligands and metal salts.

Keywords: mixed-ligand complexes; antimicrobial activity; gel electrophoresis; absorption titration; binding constant (K_b)

Introduction

Iron plays a crucial role in the survival of terrestrial organisms and participates in biochemical processes like ribonucleic reduction, energy production, photosynthesis, nitrogen reduction, oxygen transport and oxygenation.[1] The interaction between transition metal complexes and DNA has attracted many researchers due to the potential uses of these metal complexes as chemotherapeutic agents and tools in molecular biology.[2–4] Many kinds of metal complexes were synthesized in order to study their abilities to recognize and cleave to DNA.[5–7] Among them, mixed-ligand complexes are attractive reagents due to their special physiology and pharmacological activities. Many researchers have paid great attention to the synthesis, characterization and DNA cleavage abilities of metal complexes in recent years.[8–10] The intercalation of complexes with plasmid DNA pBR322 has been followed by UV spectroscopy and gel electrophoresis techniques. They provide solutions to real biological problems and guidance for detailed molecular insight into the cleavage mechanism.[11,12] In this article, we designed and synthesized mixed-ligand complexes of iron(III) with 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid (cpf) and various neutral bidentate Schiff base ligands. Mixed-ligand complexes have been used as artificial nuclease to bind and cleave plasmid pBR322 DNA.

Materials and Methods

Materials

All the chemicals used were of analytical grade. Aniline, anthranilic acid, acetophenone, acetic anhydride, 2,3-butanedione, *p*-anisidine, benzil, benzaldehyde, benzoyl chloride, hydrazine hydrate, and ferric nitrate were purchased from the E. Merck Ltd, CDH (P) Ltd, India. Ciprofloxacin hydrochloride was purchased from Bayer AG (Wuppertal, Germany). 1,8-Diaminonaphthalene was purchased from Lancaster, UK. Luria broth and agar-agar were purchased from SRL, Hi-media., India. Sperm herring DNA, sucrose, bromophenol blue, xylene cyanol FF, agarose, acetic acid and EDTA were purchased from Sigma Chemical Co., CDH (P) Ltd, India. The organic solvents were purified by recommended methods.[13]

Preparation of Schiff bases

N,N′-*Dicyclohexylidene-naphthalene-1,8-diamine* ($A^1 \approx$ *dcnd*)

An ethanolic solution (100 ml) of cyclohexanone (1.96 g, 20 mmol) was added to an ethanolic solution (100 ml) of 1,8-diamino naphthalene (1.58 g, 10 mmol). The mixture was stirred continuously for 4 h to obtain a fine yellow crystalline product. The obtained crystalline product was washed with *n*-hexane. The product was recrystallized in ethanol and dried in air. Yield, 68%; m.p., 135 °C. Found (%): C, 83.00, H, 8.09, N, 8.74. $C_{22}H_{26}N_2$ (318.45) requires (%): C, 82.97, H, 8.23, N, 8.80. Ir: 1600 (C═N), 1570 (C═C); ^{1}H NMR: 6.50–7.28 (6H, m, Ar-H), 1.51–2.37 (20H, m, Al-H); ^{13}C NMR: 173.0(C_{11} and C_{11}'), 140.0(C_1 and C_8), 22.3(C_{14}, C_{14}'), 25.3(C_{13}, C_{15}, C_{13}' and C_{15}'), 36.9(C_{12}, C_{16}, C_{12}' and C_{16}'), 117.0(C_2 and C_7), 105.9(C_9), 114.4(C_4 and C_5), 127.0(C_3 and C_6), 134.7(C_{10}).

N,N′-*Bis-(4-methoxy-phenyl)-1,2-diphenyl-ethane-1,2-diimine* ($A^2 \approx$ *bmpded*)

An ethanolic solution (100 ml) of benzil (2.10 g, 10 mmol) and *p*-anisidine (2.46 g, 20 mmol) were refluxed on water bath for

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24 h, concentrated up to its one-third volume and kept for overnight over a sulfuric acid desiccator. The obtained product was filtered, crystallized in ethanol and washed with 1 : 1 absolute ether : hexane. Yield, 64%; m.p., 120 °C. Found (%): C, 79.86, H, 5.78, N, 6.70. $C_{28}H_{24}N_2O_2$ (420.50) requires (%): C, 79.98, H, 5.75, N, 6.66. Ir: 1601 (C═N), 1574 (C═C); ^{1}H NMR: 6.65–7.91 (18H, m, Ar-H), 3.72 (6H, s, OCH_3); ^{13}C NMR: 55.3(C_{14} and C_{14}'), 113.9(C_{10}, C_{12}, C_{10}' and C_{12}'), 122.2(C_9, C_{13}, C_9' and C_{13}'), 128(C_3, C_5, C_3' and C_5'), 128.9(C_2, C_6, C_2' and C_6'),131.4(C_4 and C_4'), 134.4(C_1 and C_1'), 135.4(C_8 and C_8'), 157.3(C_{11} and C_{11}'), 165.4(C_7 and C_7').

N,N′-*Bis-(phenyl)-1,2-dimethyl-ethane-1,2-diimine* ($A^3 \approx$ *bpdmed*)

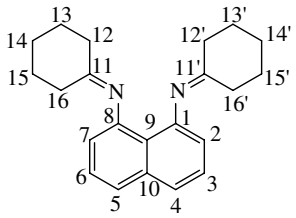
An ethanolic solution (100 ml) of aniline (1.86 g, 20 mmol) was added drop-wise to an ethanolic solution (100 ml) of 2,3-butanedione (0.86 g, 10 mmol) and refluxed on water bath for 8 h. The resulting mixture was filtered. The obtained yellow crystalline product was recrystallized in an ethanol, washed with *n*-hexane and dried in air. Yield, 58%; m.p., 114 °C. Found (%): C, 81.49, H, 6.71, N, 11.69. $C_{16}H_{16}N_2$ (236.31) requires (%): C, 81.32, H, 6.82, N, 11.85. Ir: 1613 (C═N), 1572 (C═C); ^{1}H NMR: 6.81–7.42 (10H, m, Ar-H), 2.19 (6H, s, Al-H); ^{13}C NMR: 15.4(C_1 and C_1'), 118.8(C_4, C_8, C_4' and C_8'), 123.2(C_6 and C_6'), 129.0(C_5, C_7, C_5' and C_7'), 150.9(C_3 and C_3'), 168.3(C_2 and C_2').

N,N′-*Bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine* ($A^4 \approx$ *bmpdme*)

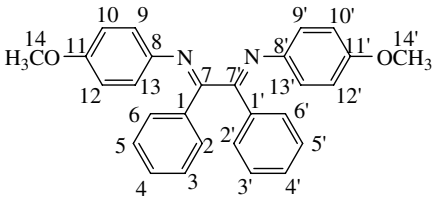
An ethanolic solution (100 ml) of *p*-anisidine (2.46 g, 20 mmol) was added drop-wise to an ethanolic solution (100 ml) of 2,3-butanedione (0.86 g, 10 mmol) and refluxed on a water bath for 8 h. The resulting mixture was filtered and the obtained yellow crystalline product was recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield, 56%; m.p., 170 °C. Found (%): C, 72.83, H, 6.64, N, 9.48. $C_{18}H_{20}N_2O_2$ (296.36) requires (%): C, 72.95, H, 6.80, N, 9.45. Ir: 1610 (C═N), 1570 (C═C); ^{1}H NMR: 6.67–6.86 (8H, m, Ar-H), 2.07 (6H, s, Al-H), 3.73 (6H, s, OCH_3); ^{13}C NMR: 15.4(C_1 and C_1'), 55.4(C_9 and C_9'), 114.2(C_5, C_7, C_5' and C_7'), 120.6(C_4, C_8, C_4' and C_8'), 144.1(C_3 and C_3'), 158.6(C_6 and C_6'), 168.9(C_2 and C_2').

3-Amino-2-phenyl-3H-quinazolin-4-one ($A^5 \approx$ *apq*)

A solution of anthranilic acid (1.37 g, 0.1mol) was prepared in pyridine (100 ml) and followed by addition of benzoyl chloride



N,*N*'-Dicyclohexylidene-naphthalene-1,8-diamine (A^1)



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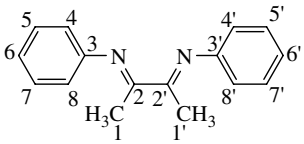

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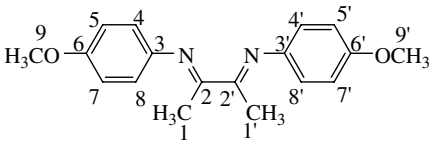
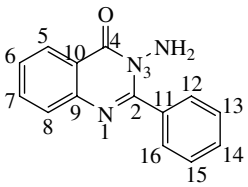
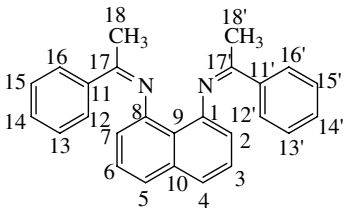


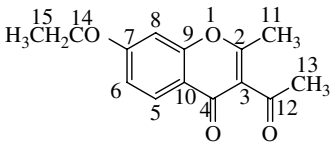
N,N'-Bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^4)



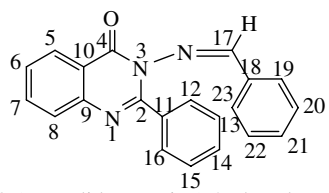
3-Amino-2-phenyl-3*H*-quinazolin-4-one (A^5)



N,*N*'-Bis-(1-phenyl-ethylidene)-naphthalene-1,8-diamine (A^6)



3-Acetyl-7-ethoxy-2-methyl-chromen-4-one (A^7)



3-(Benzylidene-amino)-2-phenyl-3*H*-quinazolin-4-one (A^8)

Scheme 1. Structures of ligands A^1–A^8.

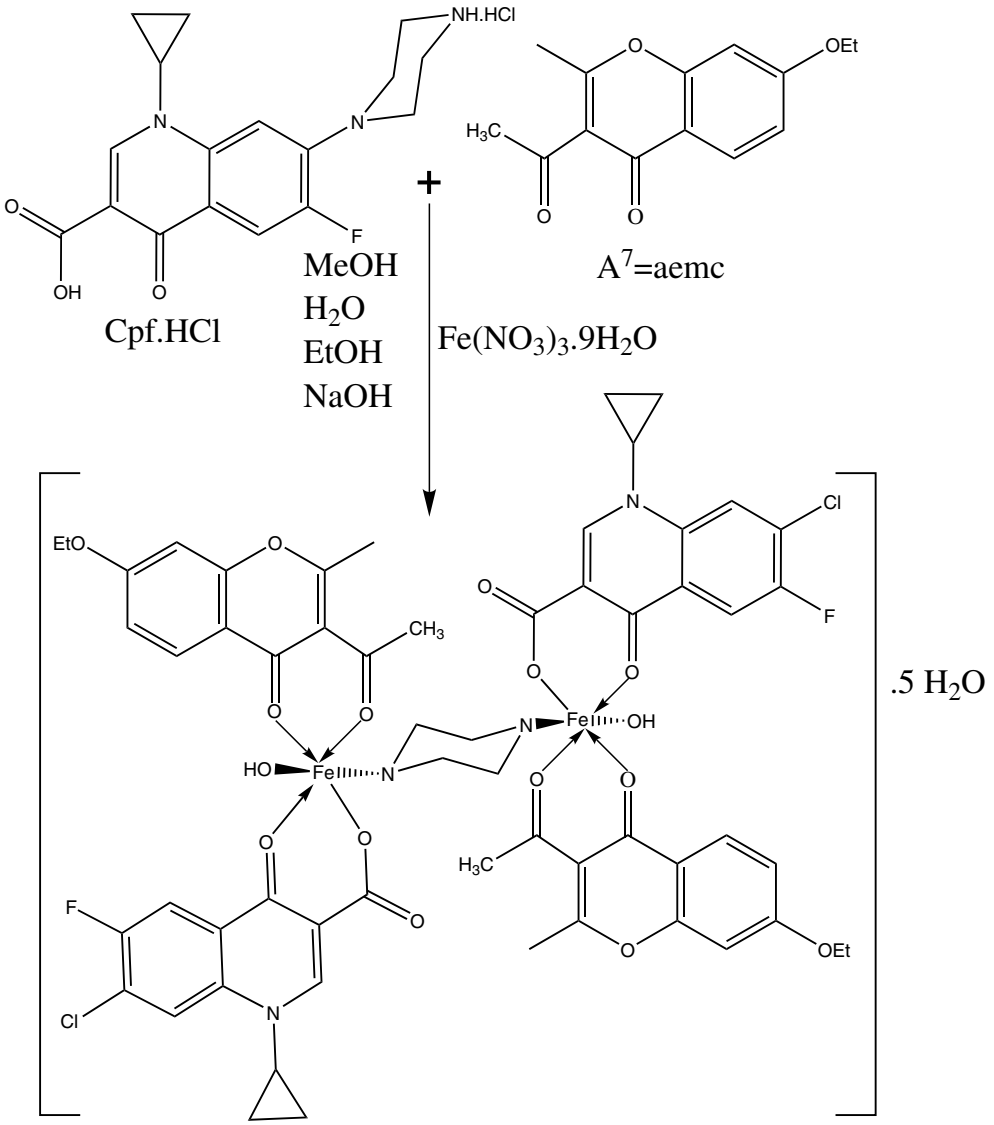
(2.814 g, 0.2mol). The resulting mixture was stirred for 0.5 h., and finally treated with 5% $NaHCO_3$ (15 ml). The separated solid was crystallized in ethanol. Yield, 80%; m.p., 120 °C. The obtained 2-phenyl-3,1-benzoxazin-4-one (0.557 g, 0.05mol) in ethanol (50 ml) and hydrazine hydrate (0.125 g, 0.05mol) in ethanol (50 ml) were mixed and refluxed for 3 h. The obtained product was crystallized in ethanol. Yield, 85%; m.p., 196 °C. Found (%): C, 70.67, H, 4.62, N, 17.59. $C_{14}H_{11}N_3O$ (237.26) requires (%): C, 70.87, H, 4.67, N, 17.71. Ir: 1680 (C═O), 1590 (C═N), 1545 (C═C); ^{1}H NMR: 7.28–8.19 (9H, m, Ar-H); ^{13}C NMR: 127.02(C_{14}), 127.5(C_{12},C_{13},C_{15} and C_{16}), 128.6(C_6 and C_8), 129.2(C_7),132.0(C_{11}), 132.2(C_5), 133(C_{10}),145.5(C_9), 165.3(C_2), 164.7(C_4).

N,N′-*Bis-(1-phenyl-ethylidene)-naphthalene-1,8-diamine (*$A^6 \approx$ *bpend)*

An ethanolic solution of (100 ml) 1, 8-diaminonaphthalene (10 mmol, 1.58 g) was added to an ethanolic solution (100 ml) of acetophenone (20 mmol, 2.34 g), refluxed on a water bath for 8 h, and kept overnight in a refrigerator. The resulting mixture was filtered. The obtained yellow crystalline product was recrystallized in ethanol, followed by washing with *n*-hexane and drying in air. Yield, 56%; m.p., 242 °C. Found (%): C, 86.20, H, 6.31, N, 7.69. $C_{26}H_{22}N_2$ (362.46) requires (%): C, 86.15, H, 6.12, N, 7.73. Ir: 1630 (C═N), 1575 (C═C); ^{1}H NMR: 7.24–7.67 (16H, m, Ar-H), 2.50 (6H, s, Al-H); ^{13}C NMR: 16.8(C_{18} and $C_{18'}$),119.39(C_9), 120.63(C_4 and C_5), 124.8(C_{14} and $C_{14'}$)125.0(C_2 and C_7), 126.73(C_{12}, C_{13}, C_{15}, C_{16}, $C_{12'}$, $C_{13'}$, $C_{15'}$ and $C_{16'}$), 128.2(C_{11} and $C_{11'}$), 136.28(C_3 and C_6), 136.45(C_{10}), 148.9(C_1 and C_8), 172.4(C_{17} and $C_{17'}$).

3-Acetyl-7-ethoxy-2-methyl-chromen-4-one ($A^7 \approx$ *aemc)*

β-Resacetophenone (10 g, 0.065mol) was heated with fused sodium acetate (10 g) in acetic anhydride (20 ml) for 3 h at 150–160 °C under anhydrous conditions. The reaction mixture was poured over crushed ice, stirred and left overnight. The separated



Scheme 2. Synthesis of $[Fe_2(L)_2(A^7)_2(OH)_2(pip)] \cdot 5H_2O$, where L = 3-acetyl-7-ethoxy-2-methyl-chromen-4-one and pip = piperazine.

product was filtered and washed with water. The obtained 3-acetyl-7-ethoxy-2-methyl-chromen-4-one was crystallized in ethanol. Yield, 56%; m.p., 127 °C. Found (%): C, 68.34, H, 5.80, $C_{14}H_{14}O_4$ (246.26) requires (%): C, 68.28, H, 5.73, Ir: 1680 (C═O), 1660 (C═O), 1550 (C═C); ^{1}H NMR: 7.17–8.24 (3H, m, Ar-H), 1.64 (3H. t, Al-H), 2.38 (3H. s, Al-H), 2.35 (2H. m, Al-H), 2.65 (2H. m, OCH_2); ^{13}C NMR: 19.79(C_{15}), 21.18(C_{11}), 32.25(C_{13}), 63.25(C_{14}), 110.78(C_8), 119.82(C_6), 121.6(C_{10}), 123.74(C_5), 127.4(C_3), 154.8(C_7), 155.8(C_9), 168.5(C_2), 175.2(C_4), 200.04(C_{12}).

3-(Benzylidene-amino)-2-phenyl-3H-quinazolin-4-one (A^8 ≈ bapq)

A methanolic solution of (100 ml) benzaldehyde (0.106 g, 0.01 mol) was added to a methanolic solution (100 ml) of 3-amino-2-phenyl-3H-quinazolin-4-one (0.237 g, 0.01 mol). The mixture was refluxed on water bath for 3 h; excess solvent was then removed under reduced pressure and kept overnight at room temperature. The resulting mixture was filtered and the obtained product was crystallized in ethanol and dried in air. Yield, 60%; m.p., 180 °C, Found (%): C, 77.60, H, 4.72, N, 13.03. $C_{21}H_{15}N_3O$ (325.36) requires (%): C, 77.52, H, 4.65, N, 12.91. Ir: 1680 (C═O), 1618 (C═N), 1584 (C═N), 1564 (C═C); ^{1}H NMR: 7.24–8.20 (14H, m, Ar-H), 8.06 (1H, s, CH═N); ^{13}C NMR: 120.8(C_{21}), 123.2(C_{14}), 127.0(C_{19}, C_{20}, C_{22} and C_{23}), 127.7(C_{12}, C_{13}, C_{15} and C_{16}), 128.9(C_6, C_8), 12 902(C_7), 132.0(C_{18}) 132.2(C_{11}), 133.0(C_5), 134.5(C_{10}), 138.7(C_9), 163.6(C_2), 164.1(C_{17}), 166.1(C_4).

The structures of ligands A^1–A^8 are shown in Scheme 1.

Preparation of mixed-ligand complexes

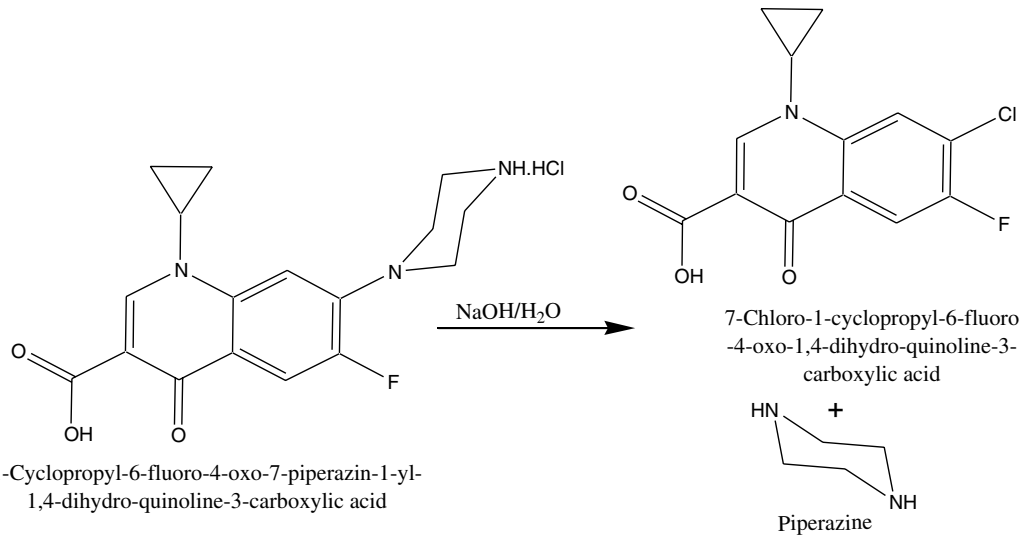
[Fe_2(Cip)$_2$(aemc)$_2$(pip)(OH)$_2$] · 5H_2O (VII)

A methanolic solution (100 ml) of $Fe(NO_3)_3 \cdot 9H_2O$ (4.04 g, 10 mmol) was added to an ethanolic solution (100 ml) *aemc* (A^7) (2.32 g, 10 mmol), followed by accumulation of a previously primed solution of Cpf · HCl (3.67 g, 10 mmol) in water; the pH was adjusted to 5.0–6.0 pH with dilute NaOH solution. The resulting reddish brown solution was refluxed for 7 h, and then heated on a steam bath to evaporate up to half the volume. The reaction mixture was kept overnight at room temperature. A fine colored product was obtained. The obtained product was washed with ether and dried over a vacuum desiccator. The proposed reaction scheme is shown in Scheme 2.

Compounds **I–VIII** were prepared according to the same method and their physicochemical parameters are summarized in Table 1.

Table 1. The physical parameter of the complexes

Compounds/empirical formula	Elemental analyses, % found (required) C	H	N	Fe(II) or Fe(III)	m.p. (°C)	Yield, %	Molecular weight
[$Fe_2(L)_2(A^1)_2(OH)_2$(pip)] · 5H_2O/$C_{74}H_{88}Cl_2F_2Fe_2N_8O_{13}$ (I)	58.39 (58.55)	5.79 (5.84)	7.24 (7.38)	7.30 (7.36)	>350	55	1518.13
[$Fe_2(L)_2(A^2)_2(OH)_2$(pip)] · 5H_2O/$C_{86}H_{84}Cl_2F_2Fe_2N_8O_{17}$ (II)	60.00 (59.98)	5.08 (4.92)	6.66 (6.51)	6.50 (6.49)	>350	61	1722.22
[$Fe_2(L)_2(A^3)_2(OH)_2$(pip)] · 5H_2O/$C_{62}H_{68}Cl_2F_2Fe_2N_8O_{13}$ (III)	55.09 (55.00)	5.12 (5.06)	8.20 (8.28)	8.32 (8.25)	250	64	1353.84
[$Fe_2(L)_2(A^4)_2(OH)_2$(pip)] · 5H_2O/$C_{66}H_{76}Cl_2F_2Fe_2N_8O_{17}$ (IV)	53.81 (53.78)	5.35 (5.20)	7.49 (7.60)	7.74 (7.58)	>350	61	1473.95
[$Fe_2(L)_2(A^5)_2(OH)_2$(pip)] · 5H_2O/$C_{58}H_{58}Cl_2F_2Fe_2N_{10}O_{15}$ (V)	51.40 (51.38)	4.26 (4.31)	10.27 (10.33)	8.33 (8.24)	240	55	1355.73
[$Fe_2(L)_2(A^6)_2(OH)_2$(pip)] · 5H_2O/$C_{82}H_{80}Cl_2F_2Fe_2N_8O_{13}$ (VI)	61.28 (61.32)	5.00 (5.02)	6.90 (6.98)	6.92 (6.95)	>350	52	1606.15
[$Fe_2(L)_2(A^7)_2(OH)_2$(pip)] · 5H_2O/$C_{60}H_{68}Cl_2F_2Fe_2N_4O_{19}$ (VII)	52.70 (52.61)	5.16 (5.00)	4.00 (4.09)	8.19 (8.15)	>350	60	1369.79
[$Fe_2(L)_2(A^8)_2(OH)_2$(pip)] · 5H_2O/$C_{72}H_{66}Cl_2F_2Fe_2N_{10}O_{15}$ (VIII)	56.56 (56.45)	4.35 (4.34)	9.16 (9.14)	7.25 (7.29)	>350	60	1531.95



Scheme 3. Proposed probable reaction.

Structural investigation

Thermogravimetric analyses and differential scanning calorimetric study were obtained with a model 5000/2960 SDTA, TA instrument (USA). Infrared spectra were recorded on an FT-IR. The ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Advance (400 MHz). Carbon, hydrogen and nitrogen elemental analyses were performed using a model 240 Perkin Elmer elemental analyzer. The diffuse reflectance spectra of the mixed-ligand complexes were recorded in the range 1700–350 nm (as MgO disks) on a Beckman DK-2A spectrophotometer. 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), and a Citizen balance. The diamagnetic correction was made using Pascal's constant.[14] The metal contents of the mixed-ligand complexes were analyzed by EDTA titration[15] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4 and HNO_3 (1 : 1.5 : 2.5). Absorption titration was carried out using Shimadzu UV–vis. spectrophotometer. 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 FBA gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. All the mixed-ligand complexes were insoluble in water, methanol and dimethyl formamide, but were soluble in 5% dimethyl sulfoxide.

Biocidal activity assay

A stock solution of 2.5 ppm was prepared by dissolving 0.25 mg of each complex in 5% DMSO solution. The biocidal test was screened by minimal inhibitory concentration (MIC). MIC was determined with the help of progressive double dilution method[16,17] in liquid media containing 1–50 ppm of the compound. The biocidal activity of the ofloxacin, levofloxacin, fluconazole, ligands, metal salts and their mixed-ligand complexes were analyzed against various Gram-negative and Gram-positive bacterial cultures of *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhi, Escherichia coli* and *Serratia marcescens* using the Agar-plate technique.[18]

DNA-binding efficacy

Absorption titration

The DNA binding study was performed on a Shimadzu UV–vis spectrophotometer. Absorption titration of compounds in 5% DMSO, and the whole system in buffer (phosphate, pH 7.2), was done by keeping fixed the amounts of iron compounds (where compound: **I** = 15.18, **II** = 17.22, **III** = 13.53, **IV** = 14.73, **V** = 13.55, **VI** = 16.06, **VII** = 13.69, **VIII** = 15.31 μg) and varying the amount of DNA, i.e. 0–10 μg. Compound–DNA solutions were employed to record absorption spectra.

Gel electrophoresis

Plasmid DNA (pBR322; 50 μM) cleavage activity of mixed-ligand complexes of Fe(III) (50 μM) was monitored using agarose gel electrophoresis. In a typical experiment, supercoiled pBR322 DNA (2.5 μg/ml) in Tris–HCl (100 mM, pH 8.0) was treated with different mixed-ligand complexes. The samples were then incubated at room temperature, and loaded with 6× loading buffer containing 40% sucrose, 0.02% bromophenol blue and 0.02% xylene cyanol FF on 0.8% agarose gel. Electrophoresis was carried out at 100 V for 1 h in TAE buffer and run in duplicate. The gel was stained

Table 2. Infrared spectral data of the complexes

Compounds	ν (C=O), cm^{-1} Pyridone	ν(COO)$_{asy}$, cm^{-1}	ν(COO)$_{sym}$, cm^{-1}	Δν, cm^{-1}	ν (C–Cl), cm^{-1}	ν (C=N), cm^{-1}, azomethine	ν (C=N), cm^{-1}, ring	ν (M–N), cm^{-1}	ν (M–O), cm^{-1}
I	1619	1592	1379	213	1136	1570	–	535	510
II	1629	1593	1382	211	1111	1579	–	540	507
III	1621	1598	1381	217	1119	1560	–	540	508
IV	1623	1600	1383	217	1140	1559	–	538	509
V	1633	1608	1388	220	1138	–	1595	540	508
VI	1629	1596	1380	216	1125	1601	–	540	510
VII	1630	1595	1381	214	1112	–	–	545	510
VIII	1620	1598	1385	213	1128	1574	1605	539	512

Table 3. Electronic spectral data of Fe(III)-complexes

Compounds	d–d transition in cm^{-1}			Charge transfer	$\mu_{eff.}$, B.M.
	$^{6}A_{1g} \rightarrow {}^{4}T_{1g}$	$^{6}A_{1g} \rightarrow {}^{4}T_{2g}$	$^{6}A_{1g} \rightarrow {}^{4}A_{1g}, {}^{4}E_{g}$		
I	19 700	23 000	25 700	35 800	5.97
II	18 800	23 800	25 750	36 700	6.02
III	19 050	23 800	25 700	36 360	6.07
IV	18 500	23 850	25 000	35 200	6.10
V	20 800	22 700	26 300	35 800	5.90
VI	19 050	21 900	25 700	36 100	6.12
VII	19 400	22 150	25 900	35 700	6.03
VIII	19 900	22 800	25 600	35 200	6.00

with ethidium bromide. The same experimental conditions were maintained in control assays. The gels were viewed on a UV transilluminator; images were captured with an attached camera and estimated using AlphaDigiDoC™ RT. Version V.4.1.0 PC-Image software.

Results and Discussion

The structural investigation of all the prepared ligands was done using elemental analyses, IR, ^{1}H and ^{13}C-NMR spectroscopy. The mixed-ligand complexes were prepared by reacting ferric nitrate with Cpf.HCl and variable ligands A^1–A^8 in a 1:1:1 ratio. The Fe(III) coordinated to deprotonated carboxylate oxygen, pyridone oxygen and N–N/N–O/O–O of neutral bidentate ligands and nitrogen of piperazine ring to form octahedral geometry. The thermal analysis suggests decomposition of crystalline water molecules and stepwise decomposition of mixed-ligand complexes. The preparation of $[Cu_2(Cip)_2(bpy)_2(pip)] \cdot 6H_2O$ and its crystal structure have been reported by Wang *et al.*[19]; they proposed possible reaction scheme for dimeric complex formation and liberation of piperazine ring from ciprofloxacin. The proposed reaction scheme is shown as Scheme 3.

All the mixed-ligand complexes are insoluble in water, ethanol, methanol, dichloromethane, chloroform, acetonitrile, hexane and DMF, while soluble in DMSO, so it is difficult to grow single crystals for X-ray diffraction analyses. The elemental analyses were in good agreement with a proposed 1:1:1; Fe(III):Cip:A^n formulation of dimeric mixed-ligand complexes.

^{1}H NMR and ^{13}C NMR spectra of the ligands

Structural analysis of the schiff bases was carried out with the help of ^{1}H NMR and ^{13}C NMR using DMSO-d_6. In the case of ^{1}H NMR spectra for the ligand, peaks around 6.5–8.2 ppm correspond to aromatic protons whereas peaks around 6.5–8.2 ppm were assigned to azomethine proton (–CH═N–). In the case of ^{13}C NMR spectra for ligands, the peak arising around 55.3 ppm was assigned to methoxy carbons, and peaks around 15.4–36.9 and 105.9–136.4 ppm were assigned to aliphatic and aromatic carbon, respectively. Peaks observed around 145.0, 165.5, 164.1 and 166.1 ppm were assigned to C–N, C═N, CH═N and C═O carbons, respectively.

IR spectra

The IR spectral data of mixed-ligand complexes are shown in Table 2. 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 towards lower energy at ~1624 cm^{-1}; suggesting that coordination occurs through the pyridone oxygen atom.[20] The absorption bands observed at 1624 and 1340 cm^{-1} in ciprofloxacin are assigned to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in mixed-ligand complexes these bands were observed at ~1598 and ~1382 cm^{-1}. The frequency separation ($\Delta\nu = \nu COO_{asy} - \nu COO_{sym}$) in investigated mixed-ligand complexes was greater than 200 cm^{-1}, suggesting that the carboxylato group has a unidentate nature.[21] The sharp band in ciprofloxacin at 3520 cm^{-1} [22] is due to hydrogen bonding, which contributes to the ionic resonance structure and peak observed because of stretching vibration of the free hydroxyl group. This band absolutely vanished in the spectra of mixed-ligand complexes, indicating deprotonation of carboxylic proton. The ν (C═O) peak for A^5, A^7 and A^8 was observed at ~1680 cm^{-1} (cyclic) and ~1660 cm^{-1} (acetyl), shifted to 1574 cm^{-1} on formation of mixed-ligand complexes.[23] These data are further supported by the ν (M–O) band's appearance at ~510 cm^{-1}. The band at about 3420 cm^{-1} for the ν_{OH} frequency indicates the coordinated hydroxo anion to iron, and is further supported by the shoulder at about 29 400 cm^{-1} in UV–vis spectra.[24] The band at ~1478 cm^{-1} was assigned to δ (C–H) bending of → N–CH_2–CH_2–N <.[22] The ν (C═N) band for A^1–A^4, A^6 and A^8 was observed at ~1612 cm^{-1}, shifted in the range 1560–1601 cm^{-1} in mixed-ligand complexes, indicating the bidentate N–N coordination of the ligand. These data are further supported by the ν (M–N) band[25] appearing at ~540 cm^{-1}.

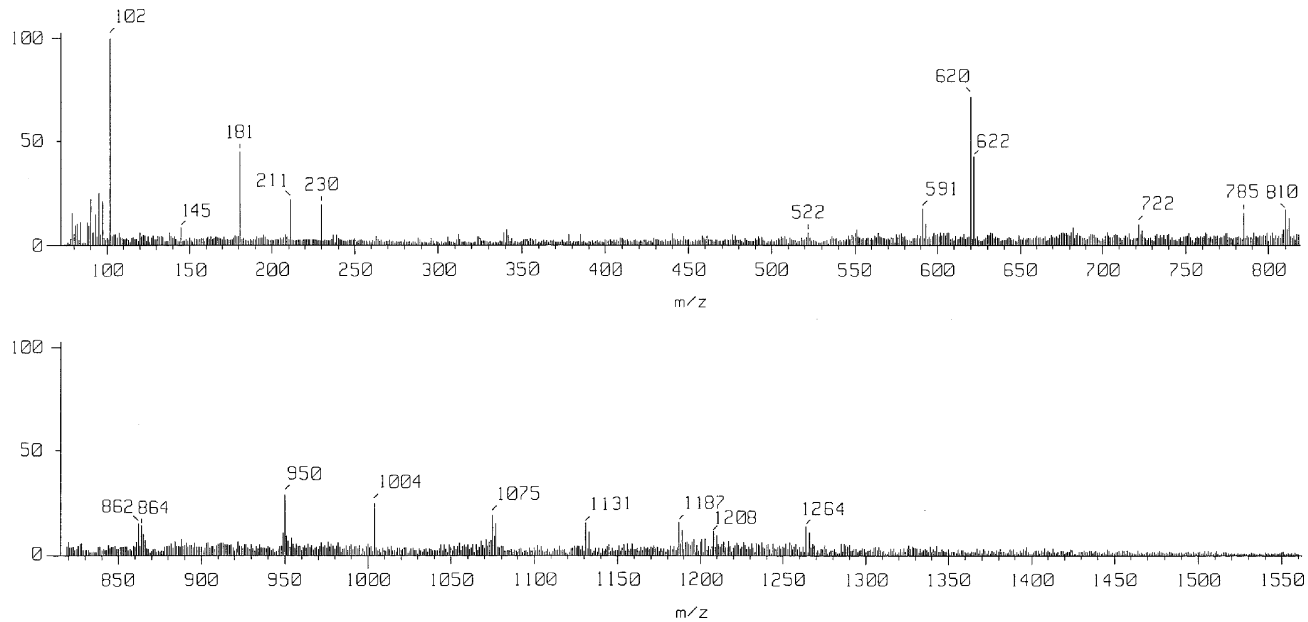


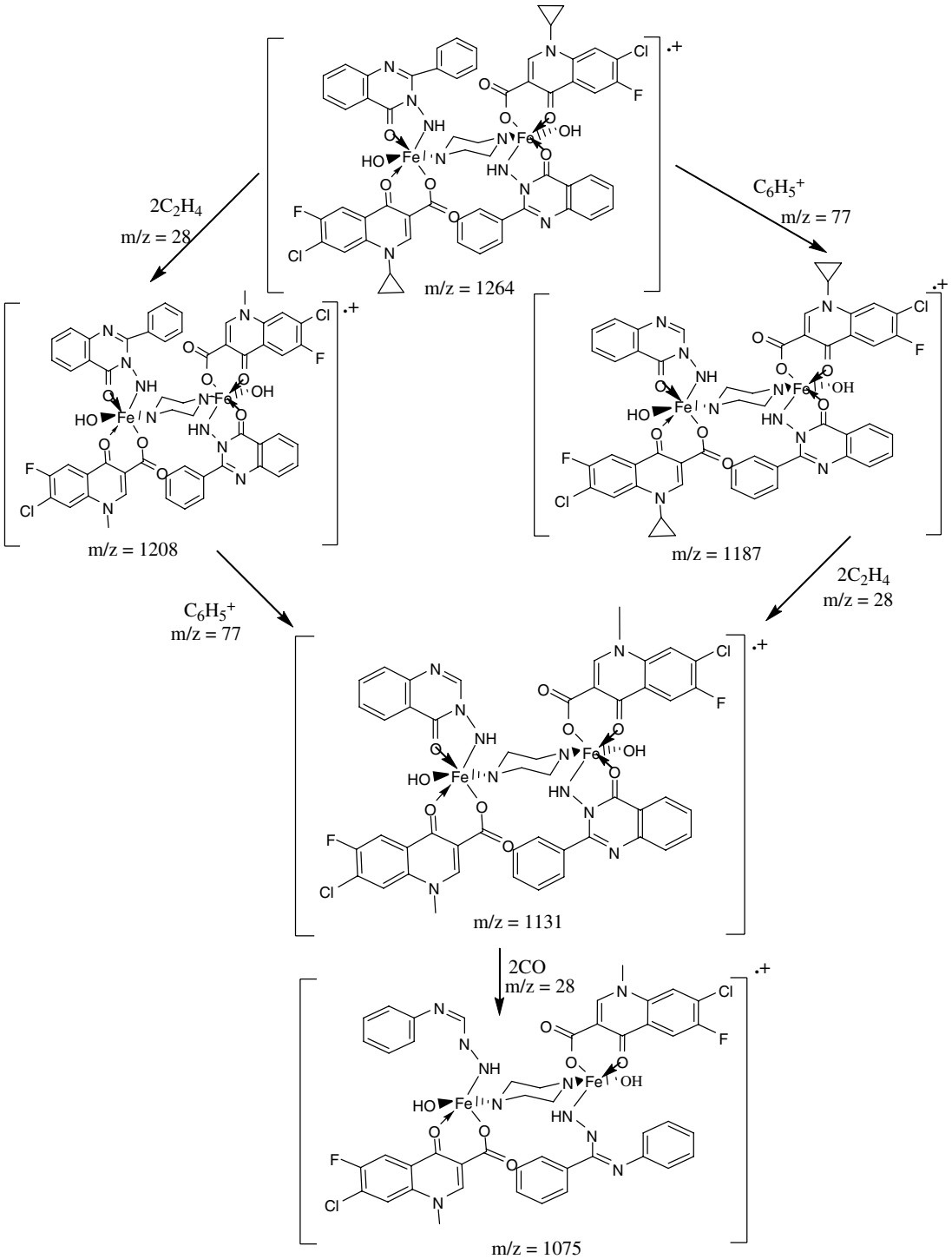
Figure 1. Mass spectra of the $[Fe_2(L)_2(A^5)_2(OH)_2(pip)] \cdot 5H_2O$.

Electronic spectra and magnetic properties

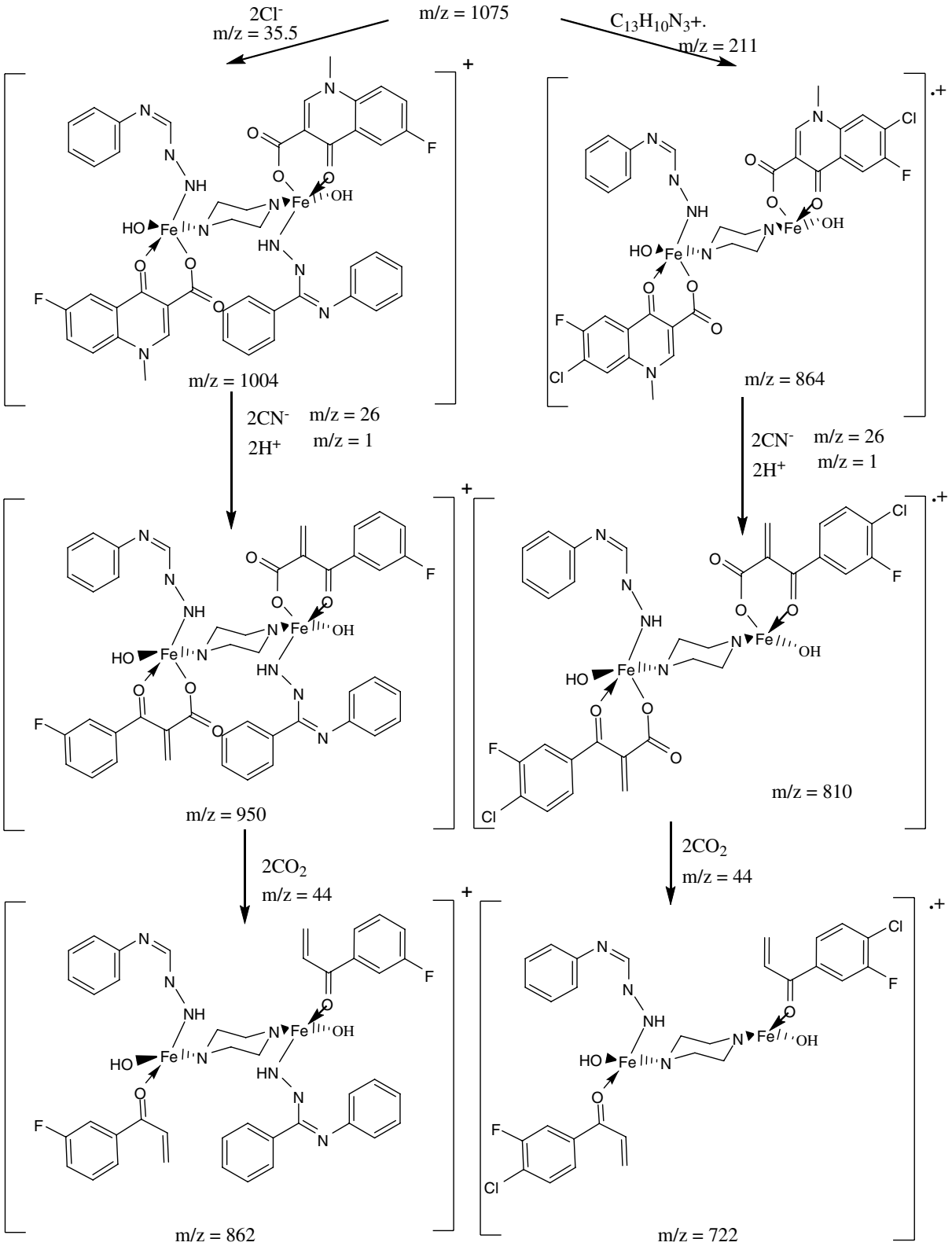
Electronic spectral data and magnetic moments for Fe(III) are presented in Table 3. The electronic spectra of mixed-ligand complexes $[Fe_2(L)_2(A^n)_2(pip)(OH)_2]\cdot 5H_2O$ exhibit three absorption bands at about ~19 400, ~23 000 and ~25 700 cm^{-1}, which may be assigned to transitions $^6A_{1g}\rightarrow{}^4T_{1g}$, $^6A_{1g}\rightarrow{}^4T_{2g}$, $^6A_{1g}\rightarrow{}^4A_{1g}$, ^4E_g, respectively.[26] The magnetic moment of $[Fe_2(L)_2(A^n)_2(pip)(OH)_2]\cdot 5H_2O$ obtained at ~6.02 B.M. is in good agreement for the six-coordinated dinuclear iron(III) system and consistent with the presence of five-unpaired electrons,[27] suggesting an octahedral geometry.

Thermogravimetric analysis

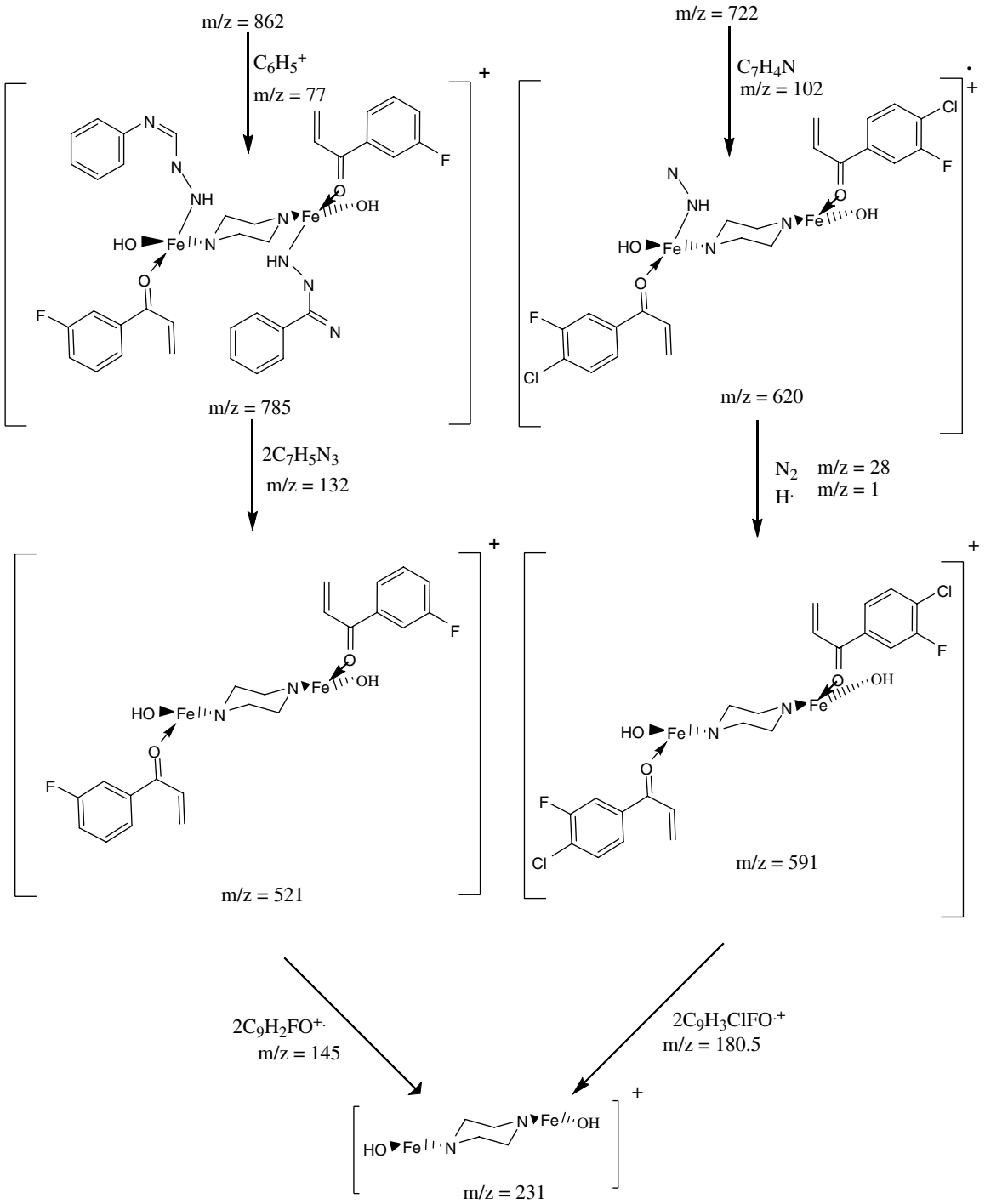
The thermogravimetric analyses for the mixed-ligand complexes were carried out within a temperature range from 20 to 800 °C in an N_2 atmosphere in order to establish their compositional



Scheme 4. Proposed fragmentation pattern of $[Fe_2(L)_2(A^5)_2(OH)_2(pip)]\cdot 5H_2O$.



Scheme 4. *Continued*.



Scheme 4. *Continued.*

differences as well as to ascertain the nature of associated water molecules.[28] The determined temperature ranges and corresponding percentage mass loss accompanying the changes in the mixed-ligand complexes on heating revealed the following things. The TG curves of Fe(III) mixed-ligand complexes show five decomposition steps. It has been observed that all the mixed-ligand complexes show a loss in weight corresponding to five water molecules in the range 50–130 °C, indicating that these water molecules are water of crystallization. The mixed-ligand complexes exhibit weight loss during 140–180 °C corresponding to two hydroxyl (OH) molecules. In the third step, weight loss during 180–220 °C corresponds to the pip. molecule, followed by liberation of L in the range 220–490 °C. Finally, decomposition of A^n occurs in the temperature range 510–710 °C and the remaining mass is in good agreement with iron oxide.

Mass spectra

The mass spectra of the $[Fe_2(L)_2(A^5)_2(OH)_2(pip)] \cdot 5H_2O$ is shown in Fig. 1. Here the peak at $m/z = 1264$ stands for the molecular

Table 4. Biocidal activity data of the compounds

Compounds	Zone of inhibition in mm					
	E. coli	*B. subtilis*	*S. aureus*	*S. typhi*	*B. cereus*	*S. marcescens*
Control	11	11	10	11	11	11
$Fe(NO_3)_2 \cdot 9H_2O$	12	17	18	15	17	17
LH(Cpf. HCl)	28	34	40	32	31	37
Std 1 (Ofl. HCl)	30	34	39	33	30	32
Std 2 (Lef. HCl)	33	36	38	29	28	34
A^1	13	15	12	11	13	14
A^2	11	11	11	14	11	12
A^3	11	11	14	12	13	11
A^4	12	12	15	11	14	12
A^5	17	18	19	16	16	17
A^6	18	12	12	17	13	14
A^7	16	16	18	15	16	17
A^8	18	19	19	18	18	19
I	30	27	31	23	25	41
II	42	40	41	28	30	38
III	38	38	37	34	35	39
IV	38	39	38	37	33	41
V	38	36	35	34	37	37
VI	43	42	31	29	29	38
VII	35	35	34	34	32	38
VIII	38	39	37	36	38	42

ion peak of complex (without water of crystallization). For several fragments there exists a peak at m, $m + 2$ and $m + 4$, indicating the presence of two Cl atoms.[29] The proposed fragmentation pattern of $[Fe_2(L)_2(A^5)_2(OH)_2(pip)] \cdot 5H_2O$ is shown in Scheme 4. The measured molecular weights were consistent with expected values.

Biocidal activity

The biocidal activity data are presented in Table 4. This increase in biocidal activity may be due to Overtone's concept,[30] chelation theory[31] or to the effect of the metal ion on the normal cell process. The activity order for each bacteria is given as below.

For E. coli: control = A^2 = A^3 < $Fe(NO_3)_2 \cdot 9H_2O$ = A^4 < A^1 < A^7 < A^5 < A^6 = A^8 < LH(Cpf. HCl) < Std 1 (Ofl. HCl) = I < Std 2 (Lef. HCl) < VII < III = IV = V = VIII < II < VI.

For B. subtilis: control = A^2 = A^3 < A^4 = A^6 < A^1 < A^7 < $Fe(NO_3)_2 \cdot 9H_2O$ < A^5 < A^8 < I < LH(Cpf. HCl) = Std 1 (Ofl. HCl) < VII < Std 2 (Lef. HCl) = V < III < IV = VIII < II < VI.

For S. aureus: control < A^2 < A^1 = A^6 < A^3 < A^4 < $Fe(NO_3)_2 \cdot 9H_2O$ = A^7 < A^5 = A^8 < I = VI < VII < V < III = VIII < Std 2 (Lef. HCl) = IV < Std 1 (Ofl. HCl) < LH(Cpf. HCl) < II.

For S. typhi: control = A^1 = A^4 < A^3 < A^2 < $Fe(NO_3)_2 \cdot 9H_2O$ = A^7 < A^5 < A^6 < A^8 < I < II < Std 2 (Lef. HCl) = VI < LH(Cpf. HCl) < Std 1 (Ofl. HCl) < III = V = VII < VIII < IV.

For B.cereus: control = A^2 < A^1 = A^3 = A^6 < A^4 < A^5 = A^7 < $Fe(NO_3)_2 \cdot 9H_2O$ < A^8 < I < Std 2 (Lef. HCl) < VI < Std 1 (Ofl. HCl) = II < LH(Cpf. HCl) < VII < IV < III < V < VIII.

For S. marcescens: control = A^3 < A^2 = A^4 < A^1 = A^6 < $Fe(NO_3)_2 \cdot 9H_2O$ = A^5 = A^7 < A^8 < Std 1 (Ofl. HCl) < Std 2 (Lef. HCl) < LH(Cpf. HCl) = V < II = VI = VII < III < I = IV < VIII.

Biocidal activity photograph for Fe(III), ligand (A^8) and its complex (VIII) given in Fig. 2.

A comparative study of biocidal activity is shown in Fig. 3. The average potency of biocidal activity was determined as well as the order of activity. Compound VIII had higher activity compared with the other compounds.

DNA cleavage

Absorption titration

Absorption spectroscopy is generally used to determine the binding of the mixed-ligand complexes with DNA. DNA binding by mixed-ligand complexes results in hypochromism. This is due to interaction between chromophores and the base pair of DNA. The extent of hypochromism is commonly consistent with the strength of intercalative interaction.[32–35] The binding of Fe(III) complexes to DNA helix has been studied through the changes in absorbance and shift in wavelength. The experiments were performed by maintaining a constant concentration of the complex while varying the nucleic acid concentration. Since the MLCT band is so weak, the IL band around 258 nm was monitored as a function of added DNA (Fig. 4). Addition of increasing amounts of DNA resulted in a slight shift in the spectra of the complexes. It is clear from the spectra that these complexes intercalate more strongly between the DNA base pairs. The intrinsic binding constants (K_b) for these complexes with pBR322 DNA were obtained using the following equation,

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

where [DNA] is the concentration of DNA in terms of nucleotide phosphate [NP], and ε_f, ε_a and ε_b correspond to the extinction

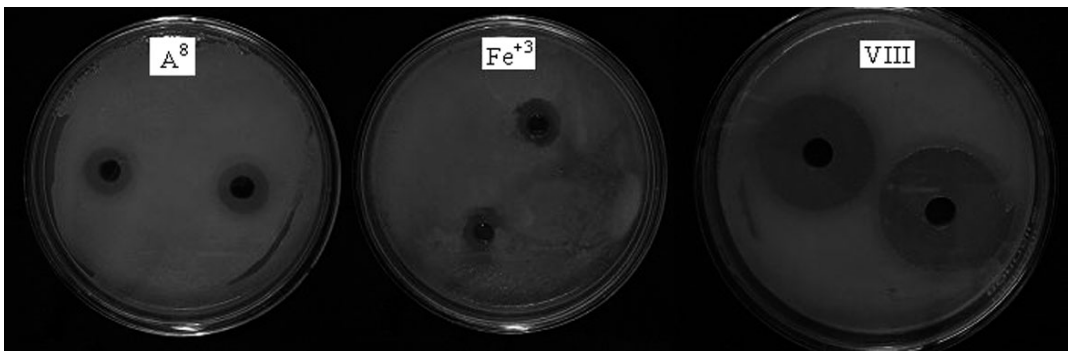


Figure 2. Biocidal activity photograph.

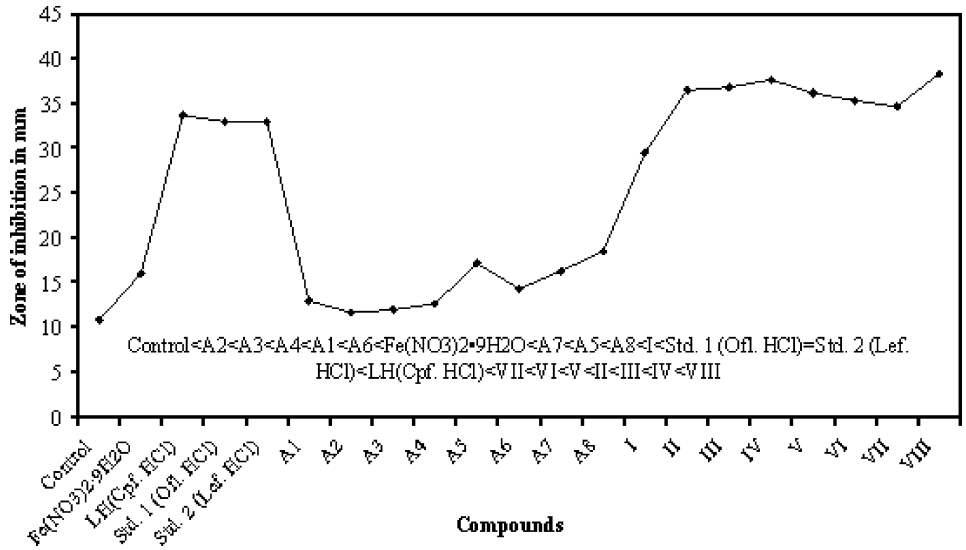


Figure 3. Comparative study of average potency of biocidal activity.

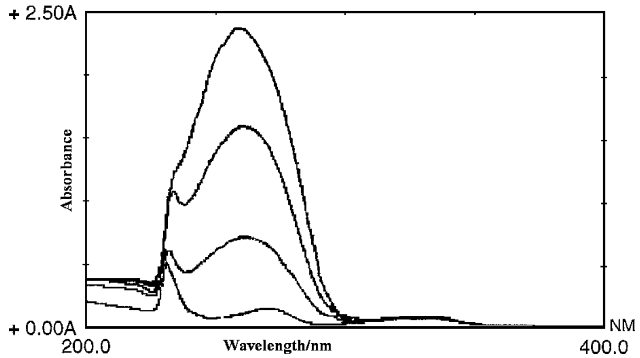


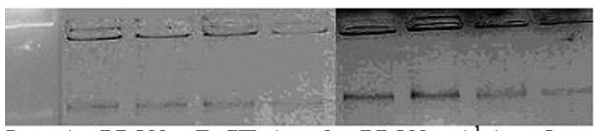
Figure 4. Absorption spectra of $[Fe_2(L)_2(A^1)_2(OH)_2(pip)] \cdot 5H_2O$ (I).

Table 5. The binding constants (K_b) of Fe(III) complexes with pBR322 DNA in Phosphate buffer pH 7.2

Complex	K_b (M^{-1})
I	3.0×10^4
II	2.0×10^4
III	2.5×10^4
IV	3.5×10^4
V	3.0×10^4
VI	4.0×10^4
VII	2.5×10^4
VIII	4.0×10^4

coefficient of the free iron complex, the extinction coefficient for each addition of DNA to the iron complex and the extinction coefficient for the iron complex in the fully bound form. In the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA], the binding constant K_b is given by the ratio of the slope to the intercept. The binding constant of Fe(III) complexes varies in the range $2.5–4.0 \times 10^4$ M^{-1} (Table 5). In general, all the constants obtained were smaller than that found for $[Ru(bpy)_2(dppz)]_2^{3+}$, which is higher than 10^6 M^{-1} [36], but

Lane 1: pBR322 (control), lane 2: pBR322 + I, lane 3: pBR322 + II, lane 4: pBR322 + III, lane 5: pBR322 + IV, lane 6: pBR322 + V, lane 7: pBR322 + VI, lane 8: pBR322 + VII, lane 9: pBR322 + VIII



Lane 1: pBR322 + Fe(III), lane 2: pBR322 + A^1, lane 3: pBR322 + A^2, lane 4: pBR322 + A^3, lane 5: pBR322 + A^4, lane 6: pBR322 + A^5, lane 7: pBR322 + A^6, lane 8: pBR322 + A^7, lane 9: pBR322 -

Figure 5. Gel of pBR322 with compounds.

comparable to those of some DNA intercalative Ru(II) complexes $1.1–4.8 \times 10^4$ M^{-1}.[37,38]

Gel electrophoresis

Mixed-ligand complexes can bind with DNA by two unique binding sites, namely groove binding and intercalation. This behavior is of great significance with regard to the relevant biological role of quinolones antibacterial in living systems. The binding of mixed-ligand complexes with supercoiled (SC) pBR322 was determined by its ability to make it bulky by changing the conformation of pBR322 DNA due to binding with reactive sites of DNA. When pBR322 was subjected to electrophoresis, the fastest migration was observed for super-coiled DNA (SC). If one strand is cleaved due to binding with reactive species, the SC form is converted in open nicked circular DNA (OC) form. Figure 5 and Table 6 show the electrophoretic process of mixed-ligand complexes, Fe(III) ion and ligands. Mixed-ligand complexes exhibit higher nuclease activity than that of Fe(III) ions and corresponding ligands.

Table 6. Gel electrophoresis data of the compounds with pBR322 DNA

Compounds	DNA %		Compounds	DNA %	
	SC	OC		SC	OC
Control	100	00	Fe(III)	66	34
A^1	84	16	IX	29	71
A^2	67	33	X	34	66
A^3	80	20	XI	51	49
A^4	74	26	XII	36	64
A^5	75	25	XIII	28	72
A^6	70	30	XIV	25	75
A^7	67	33	XV	26	74
A^8	85	15	XVI	25	75

It was observed that SC smeared on the gel while OC remained in the well. This may be due to OC becoming bulky with a high molecular weight. From the experiment we can conclude that the conversion of SC to OC is higher in the presence of mixed-ligand complexes than that of in presence of free ligands and Fe(III).

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