

## Synthesis, spectral characterization of Schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies

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MS received 1 May 2007; revised 7 July 2007

**Abstract.** A new series of transition metal complexes of Cu(II), Ni(II), Co(II), Mn(II), Zn(II), VO(IV), Hg(II) and Cd(II) have been synthesized from the Schiff base (L) derived from 4-aminoantipyrine, 3-hydroxy-4-nitrobenzaldehyde and *o*-phenylenediamine. Structural features were obtained from their elemental analyses, magnetic susceptibility, molar conductance, mass, IR, UV–Vis, <sup>1</sup>H NMR and ESR spectral studies. The data show that these complexes have composition of ML type. The UV–Vis, magnetic susceptibility and ESR spectral data of the complexes suggest a square–planar geometry around the central metal ion except VO(IV) complex which has square–pyramidal geometry. The redox behaviour of copper and vanadyl complexes was studied by cyclic voltammetry. Antimicrobial screening tests gave good results in the presence of metal ion in the ligand system. The nuclease activity of the above metal complexes shows that Cu, Ni and Co complexes cleave DNA through redox chemistry whereas other complexes are not effective.

**Keywords.** Schiff base; 4-aminoantipyrine; CT DNA; nuclease activity; DNA cleavage; antimicrobial activity.

### 1. Introduction

Schiff base of 4-aminoantipyrine and its complexes have a variety of applications in biological, clinical, analytical and pharmacological areas.<sup>1–3</sup> Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists.<sup>4,5</sup> Earlier work reported that some drugs showed increased activity, when administered as metal complexes rather than as organic compounds.<sup>6,7</sup> Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biologists. Investigations on the interaction of DNA with small molecules are important in the design of new types of pharmaceutical molecules. Since the chemical nuclease activity of transition metal complexes was discovered in the 1980s,<sup>8,9</sup> studying the interaction model and the mechanism of transition metal complexes with DNA, and exploring the application of metal complexes in antineoplastic medication, molecular biology and bioengineering have become hotspots in recent years. Some kind of metal complexes interacted with DNA could induce the

breakage of DNA strands by appropriate methods. In the case of cancer genes, after DNA strands are cleaved, the DNA double strands break. The replication ability of cancer gene is destroyed. Copper complex could cleave DNA in the presence of ascorbate or hydroquinone.<sup>11</sup> It was suggested that the reductive capability of reductants had a critical influence on DNA cleavage. The coordinating property of 4-aminoantipyrine ligand has been modified to give a flexible ligand system, formed by condensation with a variety of reagents like aldehydes, ketones thiosemicarbazides and carbazides, etc.<sup>12–22</sup> Literature search reveals that no work has been done on the condensation process of 4-aminoantipyrine, 3-hydroxy-4-nitrobenzaldehyde and *o*-phenylenediamine. In this paper we report the synthesis, characterization, redox, antimicrobial and DNA cleavage studies of transition metal complexes containing Schiff base derived from 4-aminoantipyrine, 3-hydroxy-4-nitrobenzaldehyde and *o*-phenylenediamine.

### 2. Experimental

Reagents such as 4-aminoantipyrine-3-hydroxy-4-nitrobenzaldehyde, *o*-phenylenediamine, various

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metal(II) chlorides were of Merck products, CT DNA from GENEI were used as supplied. For voltammetric experiments, tetrabutylammonium perchlorate (TBAP) (Sigma) was used as supporting electrolyte. Anhydrous grade ethanol, DMF and DMSO were purified according to standard procedures. Microanalytical data of the compounds were recorded at Central Drug Research Institute (CDRI), Lucknow. The Mass spectra of the ligand and its complexes were recorded at the Indian Institute of Technology, Mumbai.  $^1\text{H-NMR}$  spectra (300 MHz) of the samples were recorded in  $\text{CDCl}_3$  and  $\text{DMSO-}d_6$  by employing TMS as internal standard at the Madurai Kamaraj University, Madurai. IR spectra of the samples were recorded on a Perkin-Elmer 783 spectrophotometer in  $4000\text{--}400\text{ cm}^{-1}$  range using KBr pellet. The UV-Vis spectra were recorded on a Shimadzu UV-1601 spectrophotometer using DMF as a solvent. The X-band ESR spectra of the complexes were recorded at 300 and 77 K at IIT, Mumbai using TCNE (tetracyanoethylene) as the g-marker. Magnetic susceptibility of the complexes was measured by Guoy balance using copper sulphate as calibrant. Electrochemical studies were carried out using EG&G Princeton Applied Research Potentiostat/Galvanostat Model 273A, controlled by M270 software. Cyclic voltammogram was measured using a glassy carbon working electrode, platinum wire auxiliary electrode and an Ag/AgCl reference electrode. All solutions were purged with  $\text{N}_2$  for 30 min before each experiment. The molar conductance of the complexes was measured using a Systronic conductivity bridge at room temperature in DMSO. Solutions of CT DNA (calf-thymus DNA) in 50 mM NaCl/50 mM *tris*-HCl (pH = 7.2) gave a ratio of UV absorbance at 260 and 280 nm,  $A_{260}/A_{280}$  of  $\sim 1.8\text{--}1.9$ , indicating that DNA was sufficiently free of protein contamination.<sup>23</sup> DNA concentration was determined by UV absorbance at 260 nm after 1 : 100 dilutions. The molar absorption coefficient was taken as  $6600\text{ M}^{-1}\text{ cm}^{-1}$ . Stock solutions were kept at  $4^\circ\text{C}$  and used after not more than 4 days. Doubly distilled water was used to prepare the buffer. The antimicrobial activities of the ligands and their complexes were carried out by well-diffusion method.

### 2.1 Syntheses of Schiff base (L)

4-Aminoantipyrine (2.033 g, 10 mM) in 40 mL of ethanol was stirred with 3-hydroxy-4-nitrobenzaldehyde (1.671 g, 10 mM) for  $\sim 1$  h. The yellow

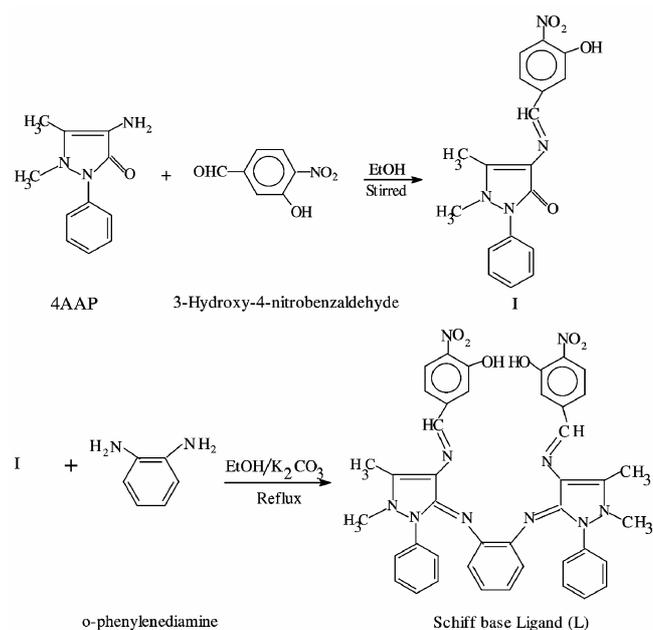
solid (I) formed was filtered and recrystallized from ethanol. Compound (I) (7.04 g, 20 mM) in 50 mL of ethanol was refluxed with *o*-phenylenediamine (1.08 g, 10 mM) for  $\sim 36$  h after adding anhydrous potassium carbonate. The potassium carbonate was filtered off from the reaction mixture and the solvent was evaporated. The red solid separated was filtered and recrystallized from ethanol (figure 1).

### 2.2 Syntheses of complexes

A solution of metal(II) chloride in ethanol (2 mM) was refluxed with an ethanolic solution of the Schiff base (2 mM) for  $\sim 5$  h. The solution was then reduced to one-third on a water bath. The solid complex precipitated was filtered, washed thoroughly with ethanol and dried *in vacuo*. The oxovanadium(IV) complex was synthesized from the sulphate salt by the same procedure but in the presence of 5 mL of 5% aqueous sodium acetate solution.

### 2.3 Antimicrobial activity

The *in vitro* biological screening effects of the investigated compounds were tested against the bacteria: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* by the well-diffusion method,<sup>24</sup> using agar nutrient as the medium. The antifungal activities of the compounds were evalu-



**Figure 1.** Formation of Schiff base ligand (L).

ated by the well-diffusion method against the fungi viz., *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola* cultured on potato dextrose agar as medium. The stock solution ( $10^{-2}$  M) was prepared by dissolving the compounds in DMSO and the solutions were serially diluted to find MIC values. In a typical procedure,<sup>25</sup> a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated 24 h for bacteria and 72 h for fungi at 35°C. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted.

#### 2.4 Gel electrophoresis

The DNA cleavage experiment was conducted using CT DNA by gel electrophoresis with the corresponding metal complex in the presence of  $H_2O_2$  as an oxidant. The reaction mixture was incubated before electrophoresis experiment at 35°C for 2 h as follows: CT DNA 30  $\mu$ M, 50  $\mu$ M each complex, 50  $\mu$ M  $H_2O_2$  in 50 mM *tris*-HCl buffer (pH = 7.2). The samples were electrophoresed for 2 h at 50 V on

1% agarose gel using *tris*-acetic acid-EDTA buffer, pH = 8.3. After electrophoresis, the gel was stained using 1  $\mu$ g/cm<sup>3</sup> ethidiumbromide (EB) and photographed under UV light using Nikon camera.

### 3. Results and discussion

The analytical data for the ligand and complexes together with some physical properties are summarized in table 1. The data from complexes correspond well with the general formula ML, where M = Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Cd(II), Hg(II) and VO(IV); L = C<sub>42</sub>H<sub>36</sub>N<sub>10</sub>O<sub>6</sub>. The magnetic susceptibilities of the complexes at room temperature were consistent with square-planar geometry around the central metal ion, except for the VO(IV) complex which shows a square-pyramidal geometry. The higher conductance values of chelates support their electrolytic nature of metal complexes.

#### 3.1 Mass spectra

The ESI mass spectra of the ligand (L) and its copper complex [CuL]Cl<sub>2</sub> recorded at room temperature were used to compare their stoichiometry composition. The Schiff base showed a molecular ion peak at

**Table 1.** Physical characterization, analytical, molar conductance and magnetic susceptibility data of the ligand and the complexes.

| Compound             | Molecular formula  | Colour     | Yield (%) | Found (Caclcd) (%) |                |              |                | Molar conductance   |                         |
|----------------------|--|------------|-----------|--------------------|----------------|--------------|----------------|---|-------------------------|
|                      |  |            |           | M                  | C              | H            | N              | $\Lambda_m^{\wedge}(\Omega^{-1} \text{cm}^2 \text{mol}^{-1})$ | $\mu_{\text{eff}}$ (BM) |
| L                    | C <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub>                   | Red        | 74        | –                  | 63.8<br>(64.9) | 4.4<br>(4.6) | 17.8<br>(18.0) | –   | –                       |
| [CuL]Cl <sub>2</sub> | CuC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Dark brown | 56        | 6.8<br>(6.9)       | 55.1<br>(55.4) | 3.8<br>(4.0) | 14.9<br>(15.4) | 42  | 1.77                    |
| [NiL]Cl <sub>2</sub> | NiC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Brown      | 54        | 6.3<br>(6.5)       | 55.4<br>(55.7) | 3.7<br>(4.0) | 15.1<br>(15.5) | 48  | –                       |
| [CoL]Cl <sub>2</sub> | CoC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Red        | 58        | 6.2<br>(6.5)       | 55.2<br>(55.7) | 3.8<br>(4.0) | 15.0<br>(15.4) | 53  | 3.62                    |
| [MnL]Cl <sub>2</sub> | MnC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Pale brown | 60        | 5.9<br>(6.1)       | 55.3<br>(55.9) | 3.9<br>(4.0) | 14.8<br>(15.5) | 44  | 5.31                    |
| [ZnL]Cl <sub>2</sub> | ZnC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Pale brown | 62        | 7.0<br>(7.2)       | 54.8<br>(55.3) | 3.7<br>(3.9) | 14.9<br>(15.3) | 56  | –                       |
| [VOL]SO <sub>4</sub> | VC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>11</sub> S               | Green      | 56        | 5.1<br>(5.4)       | 53.2<br>(53.7) | 3.5<br>(3.8) | 14.5<br>(14.9) | 36  | 1.65                    |
| [CdL]Cl <sub>2</sub> | CdC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Yellow     | 59        | 11.3<br>(11.7)     | 52.2<br>(52.6) | 3.6<br>(3.8) | 14.3<br>(14.6) | 43  | –                       |
| [HgL]Cl <sub>2</sub> | HgC <sub>42</sub> H <sub>36</sub> N <sub>10</sub> O <sub>6</sub> Cl <sub>2</sub> | Pale brown | 60        | 18.7<br>(19.1)     | 47.8<br>(48.1) | 3.2<br>(3.4) | 12.9<br>(13.4) | 48  | –                       |

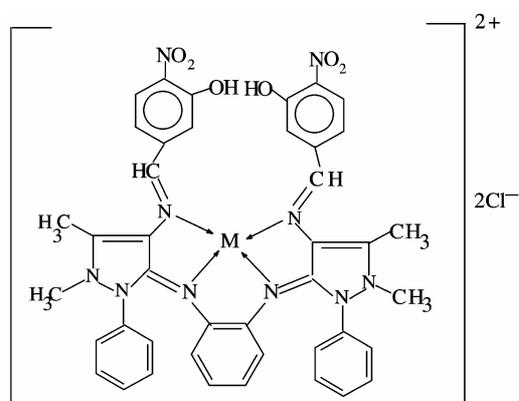
$m/z$  776 which was also supported by the 'nitrogen rule', since the compound possesses ten nitrogen atoms. The molecular ion peak for the copper complex, observed at  $m/z$  910 confirms the stoichiometry of metal chelates as ML type. It is also supported by the mass spectra of other complexes.

### 3.2 Infrared spectra

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. The IR spectra of the ligand showed a broad band in the region  $3200\text{--}3600\text{ cm}^{-1}$ , assignable to intramolecular hydrogen bonded  $\text{--OH}$  groups. The appearance of this peak in all the spectra of the complexes indicates that the  $\text{--OH}$  group is free from complexation. The spectrum of the ligand shows two different  $\text{--C=N}$  bands in the region  $1590\text{--}1550\text{ cm}^{-1}$ , which is shifted to lower frequencies in the spectra of all the complexes ( $1570\text{--}1520\text{ cm}^{-1}$ ) indicating the involvement of  $\text{--C=N}$  nitrogen in coordination to the metal ion.<sup>26,27</sup> Accordingly, the ligand acts as a tetradentate chelating agent, bonded to the metal ion via the four nitrogen ( $\text{--C=N}$ ) atoms of the Schiff base (figure 2). Assignment of the proposed coordination sites is further supported by the appearance of medium bands at  $450\text{--}400\text{ cm}^{-1}$  which could be attributed to  $\nu_{\text{M--N}}$  respectively.<sup>28,29</sup> In addition, the vanadyl complex shows a band at  $940\text{ cm}^{-1}$  attributed to  $\text{V=O}$  frequency.<sup>30</sup>

### 3.3 $^1\text{H}$ NMR spectra

The  $^1\text{H}$  NMR spectrum of the compound I, recorded in  $\text{CDCl}_3$ , showed the following signals:  $\text{=C--CH}_3$



**Figure 2.** The proposed structures of the Schiff base complexes. M = Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Cd(II), Hg(II) and VO(IV).

proton at 2.5 ppm (*s*, 3H),  $\text{N--CH}_3$  at 3.3 ppm (*s*, 3H), phenyl as multiplet at 7.2–7.8 ppm (*m*, 8H),  $\text{--CH=N--}$  at 9.7 ppm (*s*, H). Furthermore, the peak obtained at 13.3 ppm (*s*, H) is attributable to phenolic  $\text{--OH}$  group present in the 3-hydroxy-4-nitrobenzaldehyde moiety. The  $^1\text{H}$  NMR spectra of the ligand (L) and its zinc complex were recorded in  $\text{DMSO-}d_6$ . The  $^1\text{H}$  NMR spectrum of the ligand shows the following signals:  $\text{=C--CH}_3$  proton at 2.4 ppm (*s*, 6H),  $\text{N--CH}_3$  at 3.2 ppm (*s*, 6H), phenyl as multiplet at 6.9–7.5 ppm (*m*, 20H),  $\text{--CH=N--}$  at 9.8 ppm (*s*, 2H) and the peak at 13.4 ppm (*s*, 2H) is attributable to the phenolic  $\text{--OH}$  group present in the 3-hydroxy-4-nitrobenzaldehyde moiety. The presence of phenolic  $\text{--OH}$  proton noted for the Zn complex confirms the  $\text{--OH}$  proton free from complexation. The azomethine proton signal in the spectrum of the Zn complex is shifted downfield compared to the free ligand, suggesting deshielding of the azomethine group due to coordination with metal ion. There is no appreciable change in all the other signals of this complex.

### 3.4 Electronic absorption spectra

The electronic absorption spectra of the Schiff base, Cu(II), Ni(II), Co(II) and VO(IV) complexes were recorded at 300 K. The absorption region, band assignment and the proposed geometry of the complexes are given in table 2. Based on these data, a square-planar geometry has been assigned to the complexes except VO(IV) complex which has square-pyramidal geometry (figure 2). These values are comparable with other reported complexes.<sup>31–34</sup>

### 3.5 Redox studies

The cyclic voltammogram of the Cu complex in DMSO scan rate  $100\text{ mVs}^{-1}$  shows a well-defined redox process corresponding to the formation of Cu(II)/Cu(I) couple at  $E_{p_a} = 0.53\text{ V}$  and  $E_{p_c} = 0.24\text{ V}$ . This couple is found to be quasi-reversible with  $\Delta E_p = 0.29\text{ V}$  and the ratio of anodic to cathodic peak currents corresponding to a simple one-electron process.

The cyclic voltammogram for the vanadyl complex was recorded in DMSO solution. Table 3 shows two well-defined one-electron transfer redox peaks, corresponding to the formation of the VO(IV)/VO(V) and VO(IV)/VO(III) couples.<sup>35,36</sup> The peak current functions of both waves in complex are different which indicate the involvement of two different

**Table 2.** Electronic absorption spectral data of the compounds.

| Compound             | Solvent | Absorption (cm <sup>-1</sup> )   | Band assignment  | Geometry            |
|----------------------|---------|----------------------------------|--|---------------------|
| L                    | EtOH    | 40816<br>25445                   | INCT<br>INCT   | –                   |
| [CuL]Cl <sub>2</sub> | DMF     | 41493<br>26455<br>20533          | INCT<br>INCT<br><sup>2</sup> B <sub>1g</sub> → <sup>2</sup> A <sub>1g</sub>  | Square<br>Planar    |
| [NiL]Cl <sub>2</sub> | DMF     | 42016<br>26954<br>19531<br>15797 | INCT<br>INCT<br><sup>1</sup> A <sub>1g</sub> → <sup>1</sup> A <sub>2g</sub><br><sup>1</sup> A <sub>1g</sub> → <sup>1</sup> B <sub>1g</sub> | Square<br>Planar    |
| [CoL]Cl <sub>2</sub> | DMF     | 41493<br>26455<br>19607          | INCT<br>INCT<br><sup>1</sup> A <sub>1g</sub> → <sup>1</sup> B <sub>1g</sub>  | Square<br>Planar    |
| [VOL]SO <sub>4</sub> | DMF     | 41841<br>26109<br>19920<br>12315 | INCT<br>INCT<br><sup>2</sup> B <sub>2</sub> → <sup>2</sup> A <sub>1</sub><br><sup>2</sup> B <sub>2</sub> → <sup>2</sup> E                  | Square<br>Pyramidal |

**Table 3.** Cyclic voltammetric data of copper and vanadium complexes in DMSO containing 0.1 M (TBAP). Scan rate 100 mVs<sup>-1</sup>.

| Complex              | Couple         | <i>E</i> <sub>p<sub>c</sub></sub> (V) | <i>E</i> <sub>p<sub>a</sub></sub> (V) | <i>I</i> <sub>p<sub>c</sub></sub> (μA) | <i>I</i> <sub>p<sub>a</sub></sub> (μA) |
|----------------------|----------------|---------------------------------------|---------------------------------------|--|--|
| [CuL]Cl <sub>2</sub> | Cu(II)/Cu(I)   | 0.24                                  | 0.53                                  | 11.05                                  | -10.26                                 |
| [VOL]SO <sub>4</sub> | VO(IV)/VO(V)   | 0.53                                  | 0.62                                  | 15.28                                  | -15.55                                 |
|                      | VO(IV)/VO(III) | -1.03                                 | -0.57                                 | 6.75                                   | -7.44                                  |

**Table 4.** ESR spectral data of copper and vanadium complexes in DMSO at 300 and 77 K.

| Complex              | <i>A</i> <sub>  </sub> | <i>A</i> <sub>⊥</sub> | <i>A</i> <sub>iso</sub> | <i>g</i> <sub>  </sub> | <i>g</i> <sub>⊥</sub> | <i>g</i> <sub>iso</sub> |
|----------------------|------------------------|-----------------------|-------------------------|------------------------|-----------------------|-------------------------|
| [CuL]Cl <sub>2</sub> | 165                    | 55                    | 78                      | 2.38                   | 2.06                  | 2.12                    |
| [VOL]SO <sub>4</sub> | 172                    | 71                    | 104                     | 1.97                   | 2.03                  | 1.98                    |

electroactive species in solution<sup>37,38</sup> corresponding to VO(V) and VO(III).

### 3.6 ESR

The ESR spectrum of copper complex was recorded in DMSO at 300 and 77 K. The frozen solution spectrum shows a well-resolved four-line spectrum and no features characteristic for a dinuclear complex. This is also supported by the magnetic moment of Cu complex (1.77 BM) which confirms the mononuclear nature of the complex. The spin Hamiltonian parameters, calculated for the copper complex from the spectra, are given in table 4. The *g* tensor values of this copper(II) complex can be used to derive the

ground state. In square-planar complexes, the unpaired electron lies in the *d*<sub>x<sup>2</sup>-y<sup>2</sup></sub> orbital giving *g*<sub>||</sub> > *g*<sub>⊥</sub> > 2 while the unpaired electron lies in the *d*<sub>z<sup>2</sup></sub> orbital giving *g*<sub>⊥</sub> > *g*<sub>||</sub> > 2. From the observed values, it is clear that *g*<sub>||</sub> > *g*<sub>⊥</sub> > 2 suggesting that the complex is square-planar. This is also supported by the fact that the unpaired electron lies predominantly in the *d*<sub>x<sup>2</sup>-y<sup>2</sup></sub> orbital,<sup>39-42</sup> as evident from the value of the exchange interaction term *G*, estimated from the expression:

$$G = (g_{||} - 2.0023)/(g_{\perp} - 2.0023).$$

If *G* > 4.0, the local tetragonal axes are aligned parallel or only slightly misaligned. If *G* < 4.0, significant exchange coupling is present and the misalignment is appreciable. The observed value for the exchange interaction parameter for the Cu complex (*G* = 6.5) suggests that the local tetragonal axes are aligned parallel or slightly misaligned, and the unpaired electron is present in the *d*<sub>x<sup>2</sup>-y<sup>2</sup></sub> orbital. This result also indicates that the exchange coupling effects are not operative in the present complex.<sup>43</sup>

The ESR spectra of the vanadyl complex, recorded in DMSO solution at 300 and 77 K, show a typical eight-line and sixteen-line pattern respectively. The isotropic ESR parameters  $g_{\text{iso}} = 1.98$  and  $A_{\text{iso}} = 104$  can be calculated from the position spacing of the resonance lines from room temperature solution spectrum of the complex. The spectrum is like a typical eight-line pattern which shows that a single vanadium is present in the molecule which is a monomer. In the frozen solid state, the spectrum shows two types of resonance components, one set due to parallel features and the other due to perpendicular features, which indicate axially symmetric anisotropy with well-resolved sixteen-line hyperfine splitting, characteristic of an interaction between the electron and vanadium nuclear spin. From the anisotropic spectrum, the anisotropic parameters were calculated. The observed order ( $A_{\parallel} = 172 > A_{\perp} = 71$ ;  $g_{\perp} = 2.03 > g_{\parallel} = 1.97$ ) indicates that the unpaired electron is present in the  $d_{xy}$  orbital with square-pyramidal geometry around the VO(IV) chelates.<sup>44-48</sup>

### 3.7 Antimicrobial activity

For *in vitro* antimicrobial activity, the investigated compounds were tested against the bacteria *S. typhi*, *S. aureus*, *E. coli*, and *B. subtilis* and fungi *A. niger*, *A. flavus* and *R. bataicola*. The minimum inhibitory concentration (MIC) values of the investigated compounds are summarized in tables 5 and 6. The values indicate that most complexes have higher antimicrobial activity than the free ligand. Such increased activity of the metal chelates can be explained on the basis of chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand

**Table 5.** Antibacterial activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration  $\times 10^{-2}$  M).

| Compound             | <i>S. typhi</i> | <i>S. aureus</i> | <i>E. coli</i> | <i>B. subtilis</i> |
|----------------------|-----------------|------------------|----------------|--------------------|
| L                    | 5.1             | 5.3              | 5.2            | 5.5                |
| [CuL]Cl <sub>2</sub> | 4.0             | 3.8              | 4.1            | 3.9                |
| [NiL]Cl <sub>2</sub> | 4.2             | 4.1              | 4.0            | 4.2                |
| [CoL]Cl <sub>2</sub> | 4.0             | 4.5              | 4.1            | 4.4                |
| [MnL]Cl <sub>2</sub> | 4.1             | 4.4              | 4.3            | 4.6                |
| [ZnL]Cl <sub>2</sub> | 4.4             | 4.5              | 4.3            | 4.1                |
| [VOL]SO <sub>4</sub> | 3.9             | 4.0              | 4.1            | 4.2                |
| [CdL]Cl <sub>2</sub> | 4.1             | 4.3              | 4.0            | 4.2                |
| [HgL]Cl <sub>2</sub> | 4.5             | 4.4              | 4.6            | 4.3                |

orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of  $\pi$ -electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.<sup>21</sup>

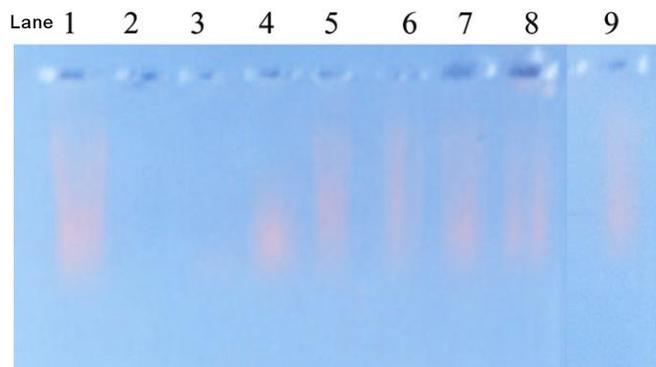
### 3.8 DNA cleavage studies

The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA-binding ability. The metal complexes were able to convert supercoiled DNA into open circular DNA. The proposed general oxidative mechanisms and account of DNA cleavage by hydroxyl radicals *via* abstraction of a hydrogen atom from sugar units that predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed.<sup>49</sup> The cleavage is inhibited by free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metal complexes bound hydroxyl radical or a peroxy species generated from the co-reactant H<sub>2</sub>O<sub>2</sub>.

In the present study, the CT-DNA gel electrophoresis experiment was conducted at 35°C using our synthesized complexes in the presence of H<sub>2</sub>O<sub>2</sub> as an oxidant. As can be seen from the results (figure 3), at very low concentration, few complexes exhibit nuclease activity in the presence of H<sub>2</sub>O<sub>2</sub>. Control experiment using DNA alone (lane 1) does not show any significant cleavage of CT-DNA even on longer

**Table 6.** Antifungal activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration  $\times 10^{-2}$  M).

| Compound             | <i>A. niger</i> | <i>A. flavus</i> | <i>R. bataicola</i> |
|----------------------|-----------------|------------------|---------------------|
| L                    | 6.5             | 6.3              | 6.4                 |
| [CuL]Cl <sub>2</sub> | 4.6             | 5.0              | 4.8                 |
| [NiL]Cl <sub>2</sub> | 4.8             | 5.2              | 5.3                 |
| [CoL]Cl <sub>2</sub> | 5.1             | 4.9              | 4.8                 |
| [MnL]Cl <sub>2</sub> | 4.8             | 5.2              | 5.0                 |
| [ZnL]Cl <sub>2</sub> | 5.2             | 4.8              | 5.1                 |
| [VOL]SO <sub>4</sub> | 4.8             | 4.9              | 5.0                 |
| [CdL]Cl <sub>2</sub> | 5.3             | 5.1              | 5.2                 |
| [HgL]Cl <sub>2</sub> | 5.1             | 5.3              | 5.4                 |



**Figure 3.** Changes in the agarose gel electrophoretic pattern of calf-thymus DNA induced by  $\text{H}_2\text{O}_2$  and metal complexes: DNA alone (lane 1); DNA +  $[\text{CuL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 2); DNA +  $[\text{NiL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 3); DNA +  $[\text{CoL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 4); DNA +  $[\text{MnL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 5); DNA +  $[\text{ZnL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 6); DNA +  $[\text{VOL}]\text{SO}_4 + \text{H}_2\text{O}_2$  (lane 7); DNA +  $[\text{CdL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 8); DNA +  $[\text{HgL}]\text{Cl}_2 + \text{H}_2\text{O}_2$  (lane 9).

exposure time. From the observed results, we conclude that the complexes, copper complex (lane 2), nickel complex (lane 3) and cobalt complex (lane 4) cleave DNA as compared to control DNA while other complexes (lane 5–9) do not cleave DNA in the presence of  $\text{H}_2\text{O}_2$ . Probably this may be due to the formation of redox couple of the metal ions and its behaviour. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage.<sup>50</sup>

#### 4. Conclusions

In this report, coordination chemistry of a Schiff base ligand, obtained from the reaction of 4-aminopyridine, 3-hydroxy-4-nitrobenzaldehyde and *o*-phenylenediamine, is described. Cu(II), Ni(II), Co(II), Mn(II), Zn(II), VO(IV), Hg(II) and Cd(II) complexes have been synthesized using the Schiff base ligand and characterized by spectral and analytical data. Based on these data, a square-planar geometry has been assigned to the complexes except VO(IV) complex which has square-pyramidal geometry. The metal complexes have higher antimicrobial activity than the ligand. The interaction of these complexes with CT-DNA was investigated by gel electrophoresis. From the observation, Cu, Ni and Co complexes cleave DNA as compared to control DNA and other complexes in the presence of  $\text{H}_2\text{O}_2$ .

#### Acknowledgements

JD thanks the Tamil Nadu Government for financial assistance. NR and AS express their sincere thanks to the University Grants Commission, New Delhi for financial assistance.

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