Hetero-metallic trigonal cage-shaped dimeric Ni₃K core complex of L-proline ligand: Synthesis, structural, electrochemical and DNA binding and cleavage activities

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Abstract. Hetero-metallic trigonal cage-shaped dimeric Ni₃K core complex of L-proline ligand has been synthesized and characterized. Single crystal X-ray diffraction analysis showed that the hetero-metallic Ni(II)–K(I) complex has a dimeric structure with nine coordinated potassium atoms and six coordinated nickel atoms. The cyclic voltammograms of the complex exhibited two successive quasireversible reduction waves at ($E_{pc}^1 = -1.02$ V and $E_{pc}^2 = -1.33$ V) and two successive irreversible oxidation waves ($E_{pa}^1 = 0.95$ V and $E_{pa}^2 = 1.45$ V) versus Ag/AgCl in DMF solution. Interaction of the complex with calf-thymus DNA (CT DNA) has been studied using spectroscopic techniques. The complex is an avid DNA binder with a binding constant of 3.6×10^8 M⁻¹. The complex showed efficient oxidative cleavage of supercoiled pBR322 DNA in the presence of the reducing agent hydrogen peroxide involving hydroxyl radical ($^{\circ}\text{OH}$) species. As evidenced from the control experiment, DNA cleavage in the presence of $^{\circ}\text{OH}$ radical was inhibited by quenchers, viz. DMSO and KI. The complex showed *in vitro* antimicrobial activity against four bacteria and two fungi and the activity is greater than that of the free ligand.

Keywords. Hetero-metallic Ni(II)–K(I) complex; X-ray crystal structure; electrochemical studies; gel electrophoresis; anti-microbial activity.

1. Introduction

In recent years, self-assemblies of coordination compounds to form polynuclear structures especially with hetero-metallic network have gained importance due to their intriguing molecular architecture and biological properties.^{1–3} Integration of alkali metal ions into hetero-metallic clusters may result in new materials with potential applications in magnetic ordering, catalytic and biological recognition.⁴ Alkali metal ions have captured more research attention owing to their low polarizability and variable coordination numbers to construct polymeric materials featuring robust structures and predominant abilities to mimic naturally occurring molecules responsible for the selective transport of ions.⁵ Literature survey reveals that chelating ligands containing O and N donor atoms have a tendency to coordinate between transition metals and alkali metals along with other bridging groups.^{6–9}

Transition metal complexes are of paramount importance for designing chemotherapeutic drugs, regulating gene expression and designing tools for molecular biology from studies on chemical modification of nucleic acids.^{10–13} Of other transition metals, nickel is a remarkably versatile metal in biological chemistry. Nickel compounds have two characteristics in common with leading antitumour drugs: direct metal binding to N7 of guanine is possible and nickel complexes are able to catalyse oxidative damage to nucleic acids.^{14–16} It was found that highly-valent nickel species may also mediate through sequence-specific oxidative cleavage of DNA by designed metallo-proteins.¹⁷ Many studies suggest that DNA is the primary intracellular target of antitumour drugs, because the interaction between small molecules and DNA can cause DNA damage in cancer cells.^{18–20} Heterocyclic bases complexes have been at the forefront of these investigations because of their unusual electronic properties, diverse chemical reactivity, and peculiar structure, which result in noncovalent interactions with DNA.^{21–25} L-proline (2-pyrrolidinecarboxylic acid) is an interesting amino

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acid and is efficient in producing polynuclear transition metal complexes; however it has rarely been used to construct polynuclear cluster incorporating alkali metal ions.

To understand the influence of alkali metal ions on the structures and properties of transition metal complexes, we report on synthesis, structure, DNA binding and cleavage activity and antimicrobial activity of hetero-metallic dimeric $\text{Ni}_3\text{-K}$ core complex of L-proline ligand. The single crystal of the complex has been structurally characterized by X-ray crystallographic analysis. The crystal structure of the complex shows that the two K^+ ions are surrounded by six Ni(II) complexes in the coordination cage provided by the three aqua ligands. The most interesting feature of this unprecedented star-shaped complex is that it is the first example of a hetero-metallic cluster in which the alkali metal cations are bridged by three water molecules with the six transition metal ions. Redox behaviour of complex was evaluated by cyclic voltammetry. The DNA binding and cleavage behaviour of the complex was evaluated using calf thymus and plasmid pBR322 DNA, respectively. Antimicrobial property of the complex was also assessed with four bacteria and two fungi.

2. Experimental

2.1 Materials and methods

All the chemicals used for synthesis were of analytical grade and were used as received without any further purification. CT DNA and pBR322 DNA were purchased from Sigma. Tris-HCl, tris-base and NaCl were purchased from Merck. Electronic spectrum of the complex was recorded on a Shimadzu UV-3101PC spectrophotometer. FT-IR spectrum was recorded in the $4000\text{--}400\text{ cm}^{-1}$ region using KBr pellets on a Bruker EQUINOX 55 spectrometer. Elemental analysis was carried out on an Elementar vario MACRO cube elemental analyser. A Biologic CHI604D electrochemical analyser was used for studying the electrochemical behaviour of complex using a three-electrode cell in which a glassy carbon electrode was the working electrode, a saturated Ag/AgCl electrode was the reference electrode and a platinum wire was used as an auxiliary electrode in nitrogen atmosphere. Concentration of the complex was 10^{-3} M in DMF and tetra(*n*-butyl)ammonium perchlorate (TBAP) (10^{-1} M) was used as the supporting electrolyte. (Perchlorate salts of metal complexes are potentially explosive and should be handled with care.)

2.2 Synthesis of complex (1)

To 0.43 g L-proline (3.7 mM) dissolved in 10 cm^3 methanol, 0.69 g $\text{Ni(ClO}_4)_2\cdot 6\text{H}_2\text{O}$ (1.85 mM) in 10 cm^3 of methanol was added slowly in drop-wise and after half an hour 0.05 g KOCN (0.62 mM) in methanol was added with constant stirring. The mixture was stirred well at room temperature for about 3 h. The dark green solution formed was then concentrated to one-third of its volume, washed well with water, methanol and ether, then dried under vacuum. The complex was then recrystallized in ethanol by slow evaporation method to obtain X-ray quality green, single crystals of complex, which appeared gradually after several days. A structure of the complex **1** is given in the line diagram. Yield: 0.27 g (53 %). M.p. ($^{\circ}\text{C}$): 310(dec.) Anal. Calc. (%) for $\text{C}_{30}\text{H}_{61}\text{Cl}_2\text{KN}_7\text{Ni}_3\text{O}_{24.50}$: C, 30.05; H, 5.10; N, 8.10. Found (%): C, 29.88; H, 4.95; N, 8.38.

2.3 X-ray diffraction analysis

Green crystals of the complex suitable for X-ray diffraction studies were obtained from slow evaporation of ethanol solution, after standing for several days. The X-ray diffraction analysis of the complex was performed on Bruker SMART APEX-II CCD diffractometer using graphite monochromated $\text{MoK}\alpha$ radiation ($0.71037\text{\AA}$). The structure was solved using direct methods and all non-hydrogen atoms were refined anisotropically by full-matrix least-square procedures (SHELX97).²⁶ Hydrogen atoms were added theoretically and refined with riding model position parameters and fixed isotropic thermal parameters.

2.4 Antimicrobial studies

Antimicrobial activity of the ligand and its complex was tested against bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) on nutrient agar medium and against fungal species (*Aspergillus niger* and *Candida albicans*) cultured on potato dextrose agar medium by disc diffusion method. For investigation of antimicrobial activity, the ligand and its complex was dissolved in dimethylsulphoxide (DMSO) to a final concentration of $100\text{ }\mu\text{g/mL}$. The sample was filled into the sterilized discs of agar plates directly, incubated at 37°C for 24 h for bacteria and 38 h for fungi. Diameter of inhibition zone around each disc was measured after incubation period and studies were performed in duplicate.

Complex 1

2.5 DNA binding studies

Binding of CT DNA with synthesized nickel(II) complex was studied using the UV absorption spectral method. Stock solution of calf thymus was prepared in 5 mM Tris-HCl/20 mM NaCl buffer (pH = 7.2), stored at 4°C. Solution of CT DNA gave a ratio of UV absorbance at 260 nm and 280 nm, A_{260}/A_{280} , of 1.8–1.9, indicating that the CT DNA was sufficiently free of proteins. Concentration of DNA was determined by the UV absorbance at 260 nm after 1:100 dilutions with a molar extinction coefficient of $6600 \text{ M}^{-1} \text{ cm}^{-1}$. Absorption titration experiments were made using different concentrations of DNA, while keeping the complex concentration constant. Stock solutions were stored at 4°C and used within 4 days. Double distilled water was used to prepare all buffer solutions.

2.6 DNA cleavage studies

The DNA cleavage studies were done by gel electrophoresis experiment for which pBR322 was used as plasmid DNA. DNA cleavage activity was evaluated by monitoring the conversion of supercoiled plasmid DNA (Sc – form I) to nicked circular DNA (Nick- form II) and linear DNA (Lin –form III). Each reaction mixture was prepared by adding 2 μL (200 ng) of supercoiled DNA, 2 μL of 500 mM Tris-HCl/500 mM NaCl buffer (pH = 7.4), 4 μL of hydrogen peroxide and 6 μL of the complex dissolved in DMF. Final reaction volume was 20 μL , final buffer concentration was 50 mM and final concentration of complex varied from 100 to 200 μM . Samples were typically incubated for 1 h at

37°C. After incubation, 5 μL of DNA loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in water) were added to each tube and the sample was loaded onto a 0.8% agarose gel in TBE buffer (89 mM Tris 89 mM borate, 1 mM EDTA pH 8.4) containing ethidium bromide (0.5 $\mu\text{g}/\text{mL}$). Negative and positive controls were loaded on each gel electrophoresis and the experiment was carried out for 1.30 h at 50 V. The reaction was also carried out in the same buffer but in the presence of hydrogen peroxide scavenger KI (20 mM) and hydroxy radical scavenger DMSO (20 mM).

3. Results and discussion

3.1 General characterizations

The complex was obtained in 52% yield by reacting nickel(II) perchlorate with L-proline ligand. The resulting complex was air-stable and characterized by elemental analysis, FT-IR and UV-vis spectral analysis. The FT-IR and UV-vis spectral data are listed in table 1. Weakness of the $\nu(\text{N-H})$ band and $\nu(\text{C=O})$ stretching vibration bands is consistent with coordination to the Ni^{2+} ion. The Ni-O and Ni-N coordination was also confirmed by the bands at 674 cm^{-1} and 560 cm^{-1} respectively.^{27,28} Electronic absorption spectrum of the complex exhibit three distinct bands due to d-d transition of high spin octahedral Ni(II).^{29,30} Solid-state reflectance spectrum of the complex also shows two bands centred at 634 nm and 372 nm indicating the presence of octahedral environment around Ni(II) in solid state.

Table 1. FT-IR and electronic spectral data of the complex (**1**).

Electronic spectra		
λ_{max} , nm (ϵ , M ⁻¹ cm ⁻¹) in DMF		FT-IR
d-d	LMCT and $\pi - \pi^*$	(ν , cm ⁻¹) (KBr Disc)
1064 (10)	374 (1350)	3426br $\nu(\text{O-H})$, 3326w $\nu(\text{N-H})$
957 (20)	299 (26060)	1587s $\nu(\text{C=O})$, 1040s $\nu(\text{ClO4})$
637 (250)		1040s $\nu(\text{ClO4})$, 674 $\nu(\text{Ni-O})$
		626 s $\nu(\text{ClO4})$, 560 $\nu(\text{Ni-N})$

3.2 Crystal structure

The complex has been structurally characterized by X-ray crystallography. A line diagram shows the structure of the complex more clearly. The ORTEP plot of crystal structure is shown in figure 1 with non-hydrogen atoms for better clarity. Crystallographic data is listed in table 2 and selected bond angle and bond distances are given in table 3. Single crystal X-ray diffraction analysis showed that the new trigonal cage-shaped heterometallic Ni(II)–K(I) complex has a dimeric structure

with nine coordinated potassium atoms and six coordinated nickel atoms. The compound crystallized in trigonal crystal system and non-centrosymmetric space group R32. As it is apparent from the figure 1, structure of the cations involves six nickel(II) building block units surrounded to two central potassium ions leading to a very unusual two triangle-shaped heterometallic Ni_3K core. Coordination sphere of the each potassium ion is nine coordination with capped square antiprismatic geometry being bonded to three oxygen atoms of three bridging aqua ligand which bridge the

Figure 1. ORTEP view of the molecular structure of **1** drawn at the 15% probability level for clarity of atoms.

Table 2. Crystallographic data and structure refinement parameters for the complex (**1**).

1	
Empirical formula	C ₃₀ H ₆₁ Cl ₂ K N ₇ Ni ₃ O _{24.50}
Formula weight	1197.991
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system, space group	Trigonal, R32
<i>a</i> (Å)	12.955(3)
<i>b</i> (Å)	12.9550(3)
<i>c</i> (Å)	47.8480(9)
α (°)	90
β (°)	90
γ (°)	120
Volume (Å ³)	6954.5(16)
Z, calculated density (mg m ⁻³)	6, 1.716
Absorption coefficient (mm ⁻¹)	1.504
<i>F</i> (000)	3738
Crystal size (mm)	0.30 × 0.20 × 0.20
Theta range for data collection (°)	2.49 to 25.81
Limiting indices, <i>h, k, l</i>	−15 ≤ <i>h</i> ≤ 12, −15 ≤ <i>k</i> ≤ 15, −57 ≤ <i>l</i> ≤ 58
Reflections collected/unique	11912/2990 [<i>R</i> (int) = 0.0296]
Completeness to $\theta = 25.81$	99.7%
Data/restraints/parameters	2990/99/227
Goodness-of-fit on <i>F</i> ²	1.076
Final <i>R</i> indices [<i>I</i> > 2σ (<i>I</i>)]	<i>R</i> 1 = 0.0522, <i>wR</i> 2 = 0.1467
<i>R</i> indices (all data)	<i>R</i> 1 = 0.0638, <i>wR</i> 2 = 0.1583
Largest difference peak/hole/ e Å ⁻³	0.720 and −0.691

Table 3. Selected bond lengths (Å) and bond angles (°) for complex (**1**).

Bond distances	(Å)	Bond angles	(°)
N(1)–Ni(1)	2.081(5)	O(4)–C(6)–K	96.8(4)
N(2)–Ni(1)#1	2.067(6)	O(5)–C(6)–K	29.1(3)
O(1)–Ni(1)	1.997(5)	C(1)–N(1)–Ni(1)	120.1(6)
O(1)–K	2.859(6)	C(4)–N(1)–Ni(1)	107.3(4)
O(3)–Ni(1)	2.138(5)	C(7)–N(2)–Ni(1)#1	110.1(4)
O(4)–Ni(1)	2.052(5)	C(10)–N(2)–Ni(1)#1	119.2(5)
O(5)–Ni(1)#1	2.055(4)	C(5)–O(1)–Ni(1)	116.0(5)
O(5)–K	2.404(5)	C(5)–O(1)–K	146.0(6)
O(8)–K	2.459(5)	Ni(1)–O(1)–K	93.3(2)
O(8)–K#2	2.459(5)	K–O(8)–K#2	94.5(3)
K–O(5)#8	2.404(5)	O(1)–Ni(1)–K	52.87(18)
K–O(5)#1	2.404(5)	O(4)–Ni(1)–K	79.43(14)
K–O(8)#8	2.459(5)	O(5)#8–Ni(1)–K	40.07(13)
K–O(8)#1	2.459(5)	N(2)#8–Ni(1)–K	121.64(16)
K–O(1)#8	2.859(6)	N(1)–Ni(1)–K	133.30(14)
K–O(1)#1	2.859(6)	O(3)–Ni(1)–K	98.92(17)
Ni(1)–O(5)#8	2.055(4)		
Ni(1)–N(2)#8	2.067(6)		

Symmetry transformations used to generate equivalent atoms:

#1 $-y+1, x-y+2, z$ #2 $x-y+1, -y+2, -z$ #3 $-y+2, x-y+2, z$ #4 $-x+y, -x+2, z$ #5 $x-y+2/3, -y+4/3, -z+1/3$ #6 $-x+y, -x+1, z$ #7 $-y+1, x-y+1, z$ #8 $-x+y-1, -x+1, z$

two potassium atoms and six oxygen atoms of six deprotonated hydroxyl oxygen of L-proline molecules.

Each nickel atom has a six-coordinate distorted octahedral environment and the six atoms coordinated to each Ni(II) ion come from two nitrogen atoms and three oxygen atoms from three different L-proline and one oxygen atom from a water molecule. It is noted that the bond distances of two trans oxygen atoms are relatively longer in the range of 2.052 and 2.138 Å than the other four distances 1.997–2.054 Å around each Ni(II). The two trans oxygen atoms of each nickel(II) come from water molecule and carbonyl oxygen of L-proline. The centred potassium atom forms perpendicular coordination (98.78°) with each Ni atom. The K–O_{water} distances range from 2.432(5)–2.514(5) Å with K...Ni distances 3.609(2)–3.648(2) Å and K–K distances of 3.613 Å. The three Ni(II) atoms of Ni₃(L-proline)₆(H₂O)₃ are bridged by six hydroxyl oxygens of L-proline with one central potassium which is embedded as one building block unit and same unit repeated through three water coordinations by having K...O distances of 2.859(6) Å. Carboxyl groups are coordinated with nickel by perpendicular position (O3–Ni1–N1 = 89.42°). Hence, three Ni atoms form cage-like formation and assisting to interact with potassium ion. In addition, two perchlorate counter ions were occupied in the crystal system and one of the perchlorates shows positional disorder with 50% occupancy site. The L-proline ligand (N1, C1/C2/C3/C4 atoms) adopts a envelope conformation which is confirmed by puckering parameters ($Q_2 = 0.3625(2)$ Å, $\varphi = 280.3(2)^\circ$)³¹ and asymmetry parameters reveals that mirror plane passing through C3 with lowest value of $7.1(1)^\circ$. Envelope confirmation and deviation from the plane occur due to short contact between H4...H2B = 2.267 Å. The least square plane deviation of atoms involving Ni1, O1, C5, C4 and N1 observed minimum parameters value of $-0.008(1)$ Å.

In the part stabilizing the molecular structure and crystal packing, there are O–H...O and C–H...O intramolecular hydrogen bonds and N–H...O, C–H...O intermolecular hydrogen bonds are involved effectively. Potential hydrogen bonds involved in the structures are also given in table S1. Two hydrogen atoms on three bridging water molecules form strong intramolecular O8–H8C...O2 hydrogen bond [O8...O2 = 2.882(1) Å; H...O2 = 2.00(5) Å; D–H...A = 169(8)] to an adjacent carbonyl oxygen atom of the L-proline ligands at $-1+y, 1+x, -z$. Since the water molecule is in crystallographic special position at z axis, it forms three pairs of O–H...O hydrogen bonds with carbonyl oxygen of L-proline and these pairs bridge the two halves of the dimeric structure. Similarly, perchlorate counter ion is involved in bridging complex structures by

C1–H1A...O9* hydrogen bond at $1-y, 2+x-y, z$. Hence, one-dimensional molecular chain runs parallel to b-axis which can be seen from the molecular packing diagram figure 2a. Another kind of one-dimensional network sheet is formed by N1–H1...O7* [O7* at $2-y, 2+x-y, z$] hydrogen bond between complex molecules (figure 2b).

3.3 Electrochemistry

Cyclic voltammogram of the complex (10^{-3} M) was recorded in dimethylformamide containing 10^{-1} M tetra(n-butyl)ammonium perchlorate (TBAP) as supporting electrolyte which is shown in figure 3. The complex showed that two successive quasi-reversible reduction processes at $E_{pc}^1 = -1.02$ V and $E_{pc}^2 = -1.33$ V. These waves can be attributed to the formation of $Ni^{II}Ni^{II}Ni^{II} \rightarrow Ni^{II}Ni^INi^I \rightarrow Ni^INi^INi^I$. Two successive irreversible oxidation waves³² are observed at $E_{pa}^1 = 0.95$ V and $E_{pa}^2 = 1.45$ V. Controlled potential electrolysis experiments indicates that the first oxidation corresponds to one electron per molecule ($n = 0.98$) and second oxidation process consumes two electrons per molecule ($n = 1.8$), when it was performed at 100 mV more negative to the second reduction peak. Hence, the redox processes can be assigned as follows:

Cyclic voltammetry was also done for the ligand (L-proline) in same condition as control experiment, which did not show any cyclic voltammetry response and hence the redox peak can be confirmed as a metal-centred response.^{33–35}

3.4 DNA binding studies

To investigate the mode of binding between the complex and the DNA double helix, UV-absorption studies were carried out. Absorption titration experiments with CT DNA show that intense absorption peaks at 234 and 298 nm in the UV region for the complex could be attributed to intraligand $\pi \rightarrow \pi^*$ transition of the coordinated groups in the complex. On addition of increasing amounts of DNA to the complex, both the two characteristic peaks decreased gradually with maximum hypochromicity of 18% and 11%, respectively, suggesting strong interaction between complex and DNA. The isobestic point at 248 nm also proved formation of the new complex between DNA and complex. Absorption peak at 234 nm increases with increase of DNA concentration, an obvious bathochromism (~ 3 nm) was found,

Figure 2. (a) View of the molecular chain of **1** showing O-H...O hydrogen bonds. (b) View of **1** with N-H...O hydrogen bond one-dimensional network layer (only those H atoms involved in the interactions are included).

Figure 3. Cyclic voltammogram of **1**; (a) oxidation process and (b) reduction process.

which demonstrated that the complex probably binds to DNA via an intercalative mode.³⁶ Spectrophotometric titration of the complex is shown in figure 4. In order to calculate quantitatively the binding strength of the complex, the intrinsic binding constant value K_b of the complex with CT DNA was determined according to eq. (1).³⁷

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

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficients ε_a , ε_f and ε_b correspond to $A_{\text{obsd}}/[\text{complex}]$, the extinction coefficient for the free complex and the extinction coefficient for the complex in the fully bound form, respectively. In plots of $[\text{DNA}]/(\varepsilon_b - \varepsilon_f)$ versus [DNA], K_b is given by the ratio of the slope to the intercept. The K_b value obtained from the absorption spectral technique for the complex was calculated as $3.6 \times 10^8 \text{ M}^{-1}$ which is relevant to that of

Figure 4. Absorption spectrum of **1** (40 μM) in 5 mM Tris-HCl/20 mM NaCl buffer at pH 7.2 in the absence and presence of increasing amounts of DNA (pathlength = 1 cm). Inset shows the least-squares fit of $[\text{DNA}]/\varepsilon_a - \varepsilon_f$ vs. [DNA] for the complex.

Figure 5. Cleavage of SC pBR322 DNA (0.2 μg , 33.3 μM) by **1** in the presence H_2O_2 (200 μM) as oxidizing agent in 50 mM Tris-HCl/50 mM NaCl buffer (pH 7.2). Lane 1, DNA control; lane 2, DNA + H_2O_2 ; lane 3, DNA + **1** (200 μM); lane 4, DNA + H_2O_2 + **1** (100 μM); lane 5, DNA + H_2O_2 + **1** (200 μM).

other typical intercalators.³⁸ The strong DNA binding nature of the complex may be due to the $\pi-\pi^*$ interactions through the heterocyclic ring of the nitrogen bases.

3.5 DNA cleavage studies

The DNA cleavage activity of the complex was investigated by agarose gel electrophoresis. Figure 5 shows the results of the gel electrophoresis separations of plasmid pBR322 DNA by the complex in the presence of H_2O_2 as a reducing agent. Cleavage activity was assessed by the conversion of supercoiled DNA (Form I) to its nicked (Form II) and linear (Form III) forms. As observed in lanes 1–3, H_2O_2 , complex alone does not

induce obvious DNA cleavage, when complex coexists with H_2O_2 (lane 4), however, prominent DNA scission is observed. More than 80% of DNA is converted from Form I to Form II by the complex at 100 μM . Conversion from Form I to Form II and Form III is completed at 200 μM for the complex (lane 5). Cleavage ability of the complex might occur due to the presence of Ni^{2+} ions which can promote the probability of double strand scission immediately after the DNA has undergone a single strand break. It is evident that the complex showed significant cleavage activity in the presence of the oxidant. This may be attributed to the formation of hydroxyl free radicals, which oxidized $\text{Ni}(\text{II})$ to $\text{Ni}(\text{III})$, presumably through Fenton-type reactions, resulting in the formation of reactive oxygen species, which could then cause oxidative damage to DNA.³⁹

Figure 6. Supercoiled pBR322 DNA (0.2 μg , 33.3 μM) treated with 200 μM complex and 200 μM H_2O_2 in the presence of different radical scavengers. Lane 1, DNA control; lane 2, DNA + H_2O_2 + 20 mM DMSO; lane 3, DNA + H_2O_2 + 20 mM DMSO + **1** (200 μM); lane 4, DNA + H_2O_2 + 20 mM KI; lane 5, DNA + H_2O_2 + 20 mM KI + **1**.

Table 4. Antimicrobial activity of the ligand and its complex (**1**).

Compound	Zone inhibition diameter (mm)					
	Bacterium				Fungi	
	Gram-negative		Gram-positive			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>C. albicans</i>
L-proline	7	5	7	6	7	7
Complex	15	16	13	15	10	15
Amikacin	17	17	17	17	NT	NT
Ketokonazole	NT	NT	NT	NT	18	18

NT–Not tested

In order to establish the DNA cleavage mechanism of the synthesized complex, the cleavage of DNA was further investigated both in the presence and absence of scavengers of oxidative species. Oxidative cleavage of plasmid DNA species may lead to the formation of hydrogen peroxide (H_2O_2), and/or hydroxy radical ($\text{HO}\cdot$) species, which cause damage to the sugar and/or base. To reveal the DNA cleavage mechanism, hydrogen peroxide scavenger (KI)⁴⁰ and hydroxy radical scavenger (DMSO) were used,⁴¹ and the results are illustrated in figure 6. In the experiment on the cleavage of DNA by **1**, no significant cleavage was observed in the presence of DMSO (lane 3) and KI (lane 5). This demonstrates that hydroxy radicals are the active oxidative species that promote DNA cleavage by **1**. In addition, DNA cleavage was dramatically enhanced in the presence of hydrogen peroxide (figure 5), which again supports the increase of hydrogen peroxide catalytic activity. Significant increase in DNA cleavage activity by **1** in the presence of H_2O_2 and inhibition of activity in the presence of DMSO and KI suggest that this reaction was preferentially proceeded by a hydroxy radical mechanism with $\cdot\text{OH}$ and hydrogen peroxide species. The oxidative reaction pathway observed here has been suggested previously in the vast majority of DNA cleavage activity.^{42–45}

3.6 Antimicrobial studies

The ligand and its nickel(II) complex were tested against four pathogenic bacteria and two fungi to test their efficacy as antimicrobial agents. The zone of inhibition values for the antimicrobial activity was given in table 4. The test results indicate that neither the ligand nor its complex showed strong activity against these organisms and consequently they are considered as mild antimicrobials. Results revealed that the nickel(II) complex have higher antimicrobial activity than the free ligand which can be explained on the basis of chelation theory.^{46–48} Chelation reduces the

polarity of ligand due to overlap of the ligand orbital and partial sharing of the positive charge of the nickel ion with donor groups. Further, it increases delocalization of π -electrons over the whole chelate ring and enhances lipophilic nature of the complex. This increased lipophilicity enhances transportation of the complex into lipid membrane and restricts further multiplicity of the microorganisms. The obtained results using the complex have also been compared with those of the standard drugs, amikacin and ketokonazole for bacteria and fungi, respectively. The complex exhibits lower activities compared with the respective standard drugs.

4. Conclusion

In this study, we have synthesized a hetero-metallic dimeric Ni_3K complex of L-proline ligands and studied its electrochemical behaviour, antimicrobial properties, DNA binding and oxidative DNA cleavage activity. Single crystal X-ray diffraction analysis of the synthesized hetero-metallic $\text{Ni(II)}\text{--K(I)}$ complex confirmed a dimeric structure with nine coordinated potassium atoms and molecular packing leading one-dimensional network chain. The electrochemical study revealed that two successive reduction processes occur at negative potential and two successive oxidation processes occur at positive potentials. The complex showed efficient DNA-binding ability and the binding constant value is consistent with other typical intercalators. The synthesized complex has significant oxidative chemical nuclease activity which could induce scission of pBR322 supercoiled DNA effectively to linear form in the presence of hydrogen peroxide as oxidizing agent and the cleavage mechanism was proceeding by a hydroxy radical mechanism with $\cdot\text{OH}$ and hydrogen peroxide species. Antimicrobial activity of the complex was found to be greater than free ligand and similar to the standard drugs.

Supplementary material

Crystallographic data in CIF format for the complex have been deposited at the Cambridge Crystallographic Data Centre, CCDC No. 891172. Copies of CIFs are available free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: -/44-1223-336-033; email: deposit@ccdc.cam.ac.uk or <http://www.ccdc.cam.ac.uk>). Table S1 can be seen in www.ias.ac.in/chemsci as Supplementary information.

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