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BINUCLEAR COPPER(II), NICKEL(II) AND OXOVANADIUM(IV) SCHIFF BASE COMPLEXES BEARING N₂O₂ DONORS AND THEIR DNA CLEAVAGE AND ANTIBACTERIAL ACTIVITY

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Abstract

Binuclear Copper(II), Nickel(II) and Oxovanadium(IV) complexes of new tetradendate Schiff base ligand derived from the condensation of 3,3',4,4'-tetraminobiphenyl with pentane-2,4-dione have been synthesized and characterized by analytical, spectral, thermal and electrochemical methods. The complexes were found to have the general formula [M₂(L)]X (where M = (Cu(II), Ni(II), and VO(IV) X=ClO₄⁻, SO₄²⁻ L = binucleating tetradendate ligand). The interaction studies of complexes with CT-DNA were carried out using cyclic voltammetry and viscosity measurements. The result shows that the complexes were bind to CT-DNA by intercalating way. The pUC18 DNA cleavage study was monitored by gel electrophoresis method. From this study, it was found that the complex cleaves pUC18 DNA in presence of the oxidant H₂O₂. The invitro antimicrobial activities of the synthesized compounds have been tested against the gram negative and gram positive bacteria. A comparative study of minimum inhibitory concentration (MIC) values of the Schiff base and its complexes indicate that the metal complexes exhibit higher antibacterial activity than the free ligand.

Keywords: Binuclear, Schiff base, Copper (II), 3,3',4,4'-tetraminobiphenyl , antimicrobial activities ,CT-DNA.

Introduction

There is a continuing interest in transition metal complexes of Schiff bases because of the presence of both hard nitrogen or oxygen and soft sulphur donor atoms in the backbones of these ligands. In the synthesis and characterization of metal complexes containing Schiff bases as ligands due to their importance as catalysts for

many reactions [1- 4]. Tetradentate Schiff base complexes are also important for designing model complexes related to synthetic and natural oxygen carriers [5]. Many transition metal Schiff base complexes have been found to considerable interesting biological properties such as antibacterial, antitumour activity [6–8]. Catalysts for various organic reactions [9], models of reaction centers of metalloenzymes [10], outstanding magnetic properties [11], and nonlinear optical materials [12]. With the complexing ability towards transition metals. Due to such a wide range of applications, new series of binuclear complexes of Copper(II), Nickel(II) and Oxovanadium(IV) ions with Schiff base ligands are of particular interest. This purpose is to alter the metal ion coordination environment, which is primarily responsible for the properties exhibited by the complex. Schiff base metal derivatives attract considerable interest and occupy an important role in the development of the chemistry of chelate systems, especially those with N_2O_2 tetradentate ligands, due to the fact that they are closely resembled to proteins. Copper and zinc are well represented in this area [13]. Copper complex could cleave DNA in the presence of ascorbate or hydroquinone [14]. It was suggested that the reductive capability of reductants had a critical influence on DNA cleavage. Several efficient cleaving agents have been developed in course of time. Nickel(II) complexes have much interest in the versatile coordination chemistry of Schiff base ligand is related to the remarkable exhibity [15-23] in the coordination geometry and the coordination number of the metal ion. Hence, we focus our interest on the 3,3',4,4'-tetraminobiphenyl containing ligand, the synthesis, spectroscopic, redox, antimicrobial and DNA cleavage studies and analytical characterizations of Copper(II), Nickel(II), and Oxovanadium(IV) complexes are reported herein. Our general strategy for preparing the desired ligand was based on the condensation of a suitable ketone precursor with aminobiphenyl. This process has been successfully applied to the tetradentate Schiff base having the mixed donor sets N_2O_2 . In this paper the synthesis of new tetradentate N_2O_2 donor type Schiff base and its metal complexes derived by the condensation of 3,3',4,4'-tetraminobiphenyl and pentane-2,4-dione is described.

Experimental

Materials and methods

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to standard procedures [24]. Metals were purchased from Merck. 3,3',4,4'-tetraminobiphenyl & pentane-2,4-dione were obtained from Aldrich. Other chemicals were also purchased from Merck and Aldrich.

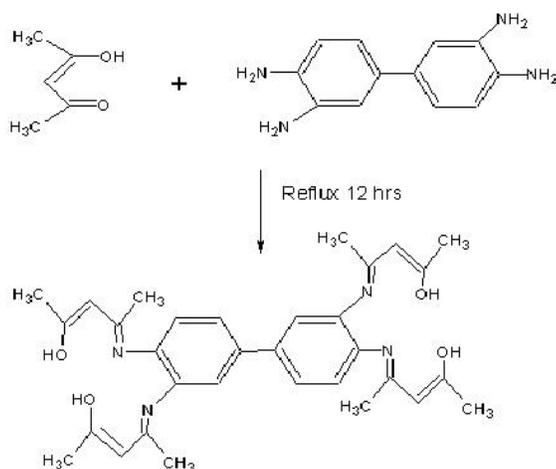
Synthesis: Caution! Perchlorate salts are potentially explosive and were handled only in small quantities with care.

Physical measurements

The elemental analysis were carried out with a Carlo-Erba 1106-model 240 Perkin Elmer analyzer. The solution conductivity measurements were performed to establish the charge type of the complexes. The complexes were dissolved in MeCN/DMF/DMSO and molar conductivities of 10^{-3} M of their solutions at 29 °C were measured. Infrared spectra were recorded on the Perkin Elmer FT-IR-8300 model spectrometer using KBr disc and Nujol mull techniques in the range of 4000-400 cm^{-1} . Electronic absorption spectra in the UV-Visible range were recorded on Perkin Elmer Lambda -25 between 200-800 nm by using DMF as the solvent. EPR spectra were recorded on a Varian JEOL-JES-TE100 ESR spectrophotometer at X-band microwave frequencies for powdered samples at room temperature. Cyclic voltammetry studies were performed on a CHI760C electrochemical analyzer in single compartmental cells at 29 °C with H₂O/DMSO (95:5) solution using tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte. TG studies were carried out in the 100-1000°C range using a NETZSCH model thermal analyzer.

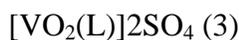
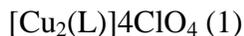
Synthesis of binucleating tetradentate Schiff base ligand

The binucleating tetradentate Schiff base was prepared by refluxing for about 12 h a mixture of 3,3',4,4'-tetraminobiphenyl (0.214 g; 1 mmol) in 20 ml of methanol, pentane-2,4-dione (0.4ml; 4 mmol) and one drop of conc.HCl in benzene (150 ml) [25]. The resulting black solid was separated, filtered under suction and dried in vacuum. The Schiff base (scheme 1) was obtained in good yield (80.0%).

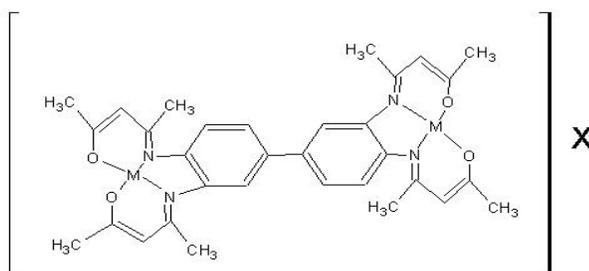


Scheme-1: Structure of binucleating tetradendate Schiff base ligand.

Synthesis of binuclear Schiff base complexes



Metal(II) perchlorates of [Cu(II), Ni(II)] and [VO(IV)] sulphate (0.2 mmol) and the potential binucleating Schiff base ligand (0.1 mmol) was dissolved in DMF (20 ml) and the mixture was heated to reflux for 3 h and the reactions were monitored by TLC. After partial evaporation of the solvent a solid 60-70% metal(II) Schiff base complexes (scheme 2) were separated and dried in vacuo. The analysis results are in good consistent with proposed formulas in Table 1.



Where, M = Cu(II), Ni(II), X = 4ClO₄⁻ ; M = VO(IV), X = 2SO₄²⁻

Scheme 2 Structure of binuclear Cu (II), Ni (II), VO (IV) Schiff base complexes

Table 1 Physical characterization, analytical data of the ligand and binuclear Schiff base complexes

Complexes	Melting Point ^a (°C)	Color	Yield (%)	Found (Calculated) (%)			
				M	C	N	H
(C ₃₂ H ₃₈ N ₄ O ₄) (Ligand)	240	black	90	-	70.85 (70.88)	10.33 (10.36)	7.01 (7.06)
[Cu ₂ (L)]4ClO ₄	260	Yellowish brown	70	12.00 (12.10)	36.02 (36.09)	5.25 (5.30)	3.56 (3.60)
[Ni ₂ (L)]4ClO ₄	248	light green	60	11.17 (11.20)	36.36 (36.40)	5.30 (5.32)	3.59 (3.63)
[VO ₂ (L)]2SO ₄	279	Dark green	70	11.75 (11.78)	44.24 (44.26)	6.45 (6.47)	4.38 (4.40)

Cyclic voltammetry

All voltammetric experiments were performed with a CHI760C electrochemical analyzer, in single compartmental cells using Tetrabutylammonium perchlorate as a supporting electrolyte. The redox behavior of the complexes have been examined in absence and in presence of CT-DNA at a scan rate 0.2 Vs⁻¹ in the potential range +1.2 to -2.0 V. A three-electrode configuration was used, comprised of a glassy carbon electrode as the working electrode, a Pt-wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. The electrochemical data such as cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}) were measured.

Viscosity experiments: Viscosity measurements were carried out from observed flow time of CT-DNA containing solution (t >100s) corrected for the flow time of buffer alone (t₀), using Ostwald's viscometer at 30 ± 0.01 °C. Flow time was measured with a digital stopwatch. Each complex was measured three times and an average flow time was calculated. Data was presented as (η / η⁰) versus binding ratio ([complex] / [DNA]) [26]

where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone. Viscosity values were calculated from the equation $\eta = t - t^0$ [27].

Gel electrophoresis

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis [15]. The gel electrophoresis experiments were performed by incubation of the samples containing 40 μM pUC18 DNA, 50 μM metal complexes and 50 μM H_2O_2 in Tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 $\mu\text{g cm}^{-3}$ ethidium bromide (EB) and photographed under ultraviolet light at 360nm. All the experiments were performed at room temperature performed at room temperature unless otherwise mentioned.

Antimicrobial activity

The *in vitro* antibacterial activity of the ligand and the complexes were tested against the bacteria *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* by well diffusion method using nutrient agar as the medium. Streptomycin was used as standard for bacteria. The stock solution (10^{-2} mol L^{-1}) was prepared by dissolving the compound in DMF and the solution was serially diluted in order to find minimum inhibitory concentration (MIC) values. In a typical procedure, a well was made on the agar medium inoculated with microorganisms in a Petri plate. The well was filled with the test solution and the plate was incubated for 24 h for bacteria at 35 °C. During the 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.

Results and discussion

The binuclear Schiff base complexes of the type $[\text{M}_2(\text{L})]\text{X}$ (where $\text{M} = (\text{Cu}(\text{II}), \text{Ni}(\text{II}), \text{and VO}(\text{IV}))$ $\text{X} = \text{ClO}_4^-, \text{SO}_4^{2-}$ ($\text{L} =$ binucleating Schiff base ligand) (scheme 2) were achieved by reacting transition metals $\text{Cu}(\text{II})$, $\text{Ni}(\text{II})$, and $\text{VO}(\text{IV})$ with binucleating tetradentate Schiff base ligand. The Schiff base ligand has been synthesized from pentane-2, 4-dione and 3,3',4,4'-tetraminobiphenyl (scheme 1) ($\text{C}_{32}\text{H}_{38}\text{N}_4\text{O}_4$), in 2:1 mole ratio. The analysis of the complexes were consistent with the stoichiometry proposed and are summarized in Table 1. The

data in consistent with the earlier reports support the proposed formulation of the binuclear complexes (scheme 2). The higher conductance values (Table 2) of chelates support the electrolytic (1:2) nature of metal complexes. The new binuclear complexes are stable, hygroscopic with higher melting points, insoluble in water, soluble in acetonitrile, chloroform, DMF and DMSO.

Table-2: Molar conductance data of the binuclear Schiff base the complexes.

Complexes	Solvent	Molar conductance	Types of
		Λ_m (ohm ⁻¹ cm ² mol ⁻¹)	electrolyte
	MeCN	270	1:2
[Cu ₂ (L)]4ClO ₄	DMF	230	1:2
	MeCN	110	1:2
[Ni ₂ (L)]4ClO ₄	DMSO	125	1:2
	MeCN	144	1:2
[VO ₂ (L)]2SO ₄	DMSO	132	1:2

IR spectra

The important IR absorption frequencies of the synthesized complexes are shown in Table 3. The azomethine nitrogen $\nu_{C=N}$ stretching frequency of the free ligand appears around 1624 cm⁻¹, which is shifted to lower frequencies in the spectra of all the complexes (1595-1605 cm⁻¹). These bands are shifted to lower wave numbers indicating the involvement of azomethine nitrogen in coordination to the metal ion [28]. The above i.r. data clearly indicate that the carbonyl groups of pentane-2, 4-dione have reacted with the amine groups of 3,3',4,4'-tetraminobiphenyl through the condensation of the metal atoms. The ligand displays $\nu_{C=O}$ absorptions at 1270 cm⁻¹. On complexation this band is shifted to higher frequency in the range of 1295-1310 cm⁻¹ for all the Schiff base complexes which suggest that the carbonyl group is involved in coordination in the enol form through deprotonation [29]. This is further supported by the disappearance of the ν_{OH} in the range of 3400-3440 cm⁻¹ in all

the complexes . Accordingly, the ligand acts as a tetradentate chelating agent bonded to the metal ion via two –C–O groups & two –C=N azomethine nitrogen atoms of the Schiff base (scheme 2). Assignment of the proposed coordination sites is further supported by the appearance of medium bands at 500-540 cm⁻¹ and 440-470 cm⁻¹ which could be attributed to M-O, M-N respectively [30]. In addition, the Oxovanadium complexes shows a band at 960 cm⁻¹- 980 cm⁻¹ attributed to V=O stretching frequency [31]. A further examination of Infrared spectra of complexes shows the presence of a band in the 1110-1130 cm⁻¹ region. The strong band is ascribable to ClO₄⁻ & SO₄²⁻ ions [32].

Table-3: Infrared spectral data for the ligand and binuclear Schiff base complexes.

Complexes	$\nu(-C-O)^a$	$\nu(C=N)^a$	$\nu(-OH)^a$	$\nu(V=O)^a$	$\nu(M-O)^a$	$\nu(M-N)^a$	ClO ₄ ⁻ /SO ₄ ²⁻ ^a
(C ₃₂ H ₃₈ N ₄ O ₄)							
(Ligand)	1270	1624	3440	-	-	-	-
[Cu ₂ (L)]4ClO ₄	1295	1595	3415	-	540	450	1118
[Ni ₂ (L)]4ClO ₄	1306	1605	3435	-	515	470	1130
[VO ₂ (L)]2SO ₄	1310	1600	3400	980	500	440	1110

^a Units in cm⁻¹

Electronic spectra

The UV-visible spectra are often very useful in the evaluation of results furnished by other methods of structural investigation. The electronic spectral measurements were used for assigning the stereochemistries of metal ions in the complexes based on the positions and number of d-d transition peaks. The electronic absorption spectra of the Schiff base ligand and its complexes were recorded in DMF solution in the range of 200 to 800 nm regions and the data are presented in Table 4. The absorption spectrum of free ligand consist of an intense bands

centered at 340 nm attributed to $n-\pi^*$ transitions of the azomethine group. Another intense band in higher energy region of the spectra of the free ligand was related to $\pi\rightarrow\pi^*$ transitions of benzene rings. These transitions are also found in the spectra of the complexes, but they shifted towards lower frequencies, confirming the coordination of the ligand to the metal ions. Further, the d-d transition of the complex showed a broad band centered at 560 nm for Cu(II) complex Fig. 1. This is due to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition [33]. The spectra of Ni(II) complex in the visible region at about 500 and 485 nm is assigned to ${}^1A_{1g} \rightarrow {}^1A_{2g}$, ${}^1A_{1g} \rightarrow {}^1B_{1g}$, transitions, suggesting an approximate square planar geometry of the ligand around the metal ions [34]. The intense charge transfer band at 520-540 nm in Oxovanadium(IV) complex assigned to ${}^2B_2 \rightarrow {}^2A_1$, ${}^2B_2 \rightarrow {}^2E$ transitions. This is due to electron delocalization over whole molecule on complexation. Based on these data, a square planar geometry has been assigned to the complexes except VO(IV) complex which has square pyramidal geometry. These values are comparable with other reported complexes [35].

Fig. 1 Absorption spectra of the binuclear Cu(II) Schiff base complex $[Cu_2(L)]ClO_4$.

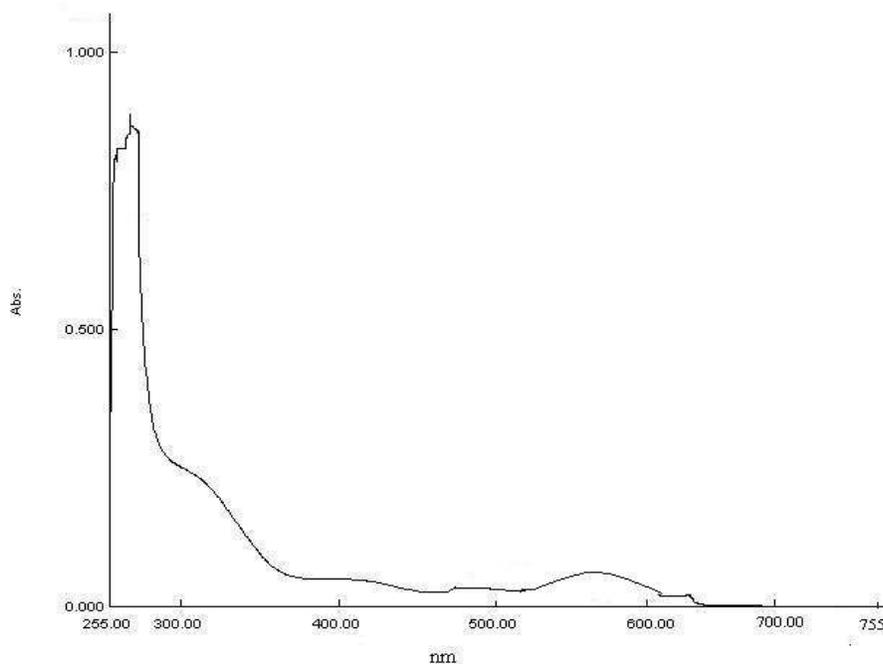


Table-4: Absorption spectral data of the ligand and binuclear Schiff base complexes.

Complexes	Absorption (λ_{\max})(nm)			
	d-d	$\pi \rightarrow \pi^*$ Benzene/ imino	$n \rightarrow \pi^*$ Azomethine	L \rightarrow MCT
	(C ₃₂ H ₃₈ N ₄ O ₄) Ligand	-	263,255	342
[Cu ₂ (L)]4ClO ₄	560	279,239	330	430
[Ni ₂ (L)]4ClO ₄	500	272,253	334	375
[VO ₂ (L)]2SO ₄	540	278,245	324	420

ESR spectrum

ESR studies of paramagnetic transition metal(II) complexes yield information about the distribution of the unpaired electrons and hence about the nature of the bonding between the metal ions and its ligand. The X-band ESR spectrum of the copper complex was recorded on a powder solid at room temperature Fig. 2. It exhibits an axial signal which can be interpreted in terms of tetrahedral species with a strong signal in the low field region, corresponding to $g_{\perp} = 2.08$, and a weak signal in the high field region due to $g_{\parallel} = 2.16$ while the g_{iso} is 2.07. Splitting of the signal in the high field region may be due to a difference in surroundings between the two copper(II) centers suggesting a binuclear structure for this complex [36]. As seen in Table 5, the presence of $g_{\parallel} > g_{\perp}$ is evidence for square planar geometry around copper(II) atom [37]. The axial symmetry parameter (G) value of Cu(II) complex (less than 4) show that the exchange interaction is negligible. The Cu(II) complex is dimer with the unpaired electron lies in the $d_{x^2-y^2}$ orbital. The pairing of electrons is prevented by the distance

between two Cu(II) atoms provided by the bridging bis-tetradentate Schiff base ligand. Its interesting to note that the $g_{\parallel} > g_{\perp} > 2.0023$, indicating that the ground state of Cu(II) is predominantly $d_{x^2-y^2}$. The observed values of Vanadyl complex $g_{\parallel} = 2.02 > g_{\perp} = 1.99$ indicates that the unpaired electron is present in the d_{xy} orbital with square pyramidal geometry around the VO(IV) chelates [38].

Fig-2: X-band ESR spectra of binuclear Cu(II) Schiff base complex [Cu₂(L)]ClO₄ at room temperature.

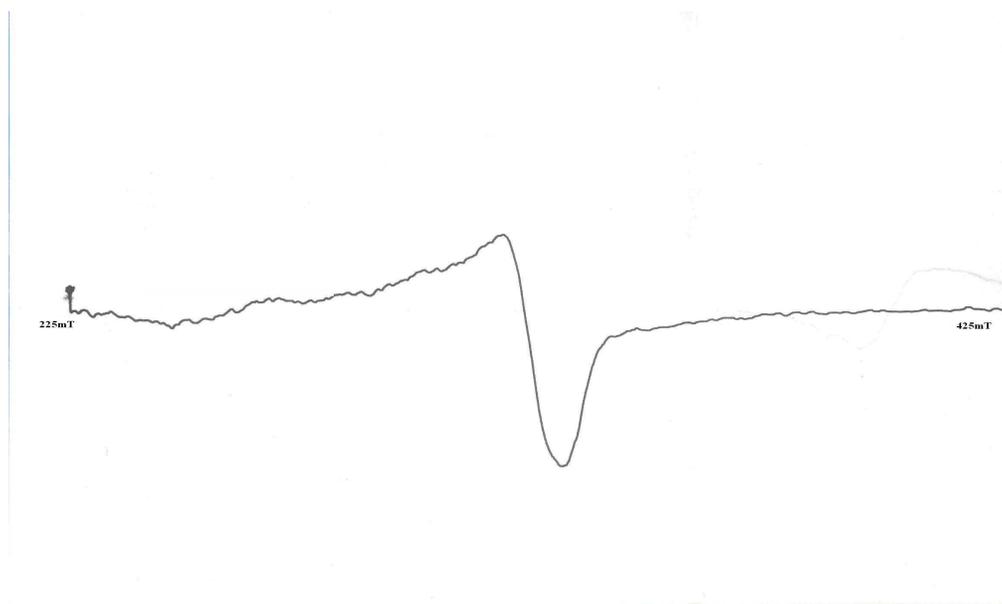


Table-5: ESR spectral data of the binuclear Schiff base complexes.

Complexes	g_{\parallel}	g_{\perp}	g_{iso}	G
[Cu ₂ (L)]4ClO ₄	2.16	2.08	2.07	2.03
[VO ₂ (L)]2SO ₄	2.02	1.99	2.02	-1.44

Cyclic voltammetry studies

Cyclic voltammetry is the most versatile electroanalytical technique for the study of electroactive species. The important parameters of a cyclic voltammogram are the magnitudes of the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). Cyclic voltammetry has been employed to study the interaction of complex with CT-DNA. The cyclic voltammogram of complex 1 in the absence of CT-DNA shows in Fig. 3(a) reveals non-Nernstian but fairly reversible/quasi-reversible one electron redox process involving Cu(II)/Cu(I) couple. The cyclic voltammograms of complexes were obtained in H₂O/DMSO (95:5) solution, at scan rate 0.2 Vs⁻¹ over a potential range from +1.2 to -2.0 V. In the absence of CT-DNA, complex 1 and other complexes data are listed in Table 6. the anodic peak potential (E_{pa}) of complex 1 appeared at -0.412 V and the cathodic (E_{pc}) at -1.198 V. The cyclic voltammograms of complex 1 reveals a one electron quasireversible wave attributed to the redox couple Cu(II)/Cu(I) with the formal electrode potential, $E^0 = -1.610$ V, the ratio of anodic peak current to cathodic peak current (I_{pa} / I_{pc}) is 0.464 and $\Delta E_p = +0.786$ V which is larger than the Nernstian value observed for the one electron transfer couple. On addition of CT-DNA, the complex 1 Fig. (3b) shows a shift in $E_{pc} = (-1.121$ V), $E_{pa} = (-0.452$ V) and $\Delta E_p = (0.669$ V) values indicating strong binding of binuclear complex with CT-DNA. The decrease in ratio of anodic to cathodic peak currents from 0.464 to 0.322 signify that adsorption of Cu(I) is enhanced in the presence of CT-DNA. Further, the shift in E^0 value and increase in peak heights potentials suggest that both Cu(II) and Cu(I) form of complex 1 bind to CT-DNA [39]. The ratio of equilibrium constants K_+/K_{2+} for the binding of the Cu(II) and Cu(I) forms of complex 1 to CT-DNA have also been calculated using the following equation (Scheme 3).

$$E_b^0 - E_f^0 = 0.059 \log (K_+/K_{2+}) \rightarrow (2)$$

Where E_b^0 and E_f^0 are the formal potentials of the Cu(II)/Cu(I) couple in the free and bound species respectively. The ratio of binding constant is equal to 1, K_+/K_{2+} Suggesting that both Cu(II) and Cu(I) forms interact with DNA to the same extent [40].

Scheme 3

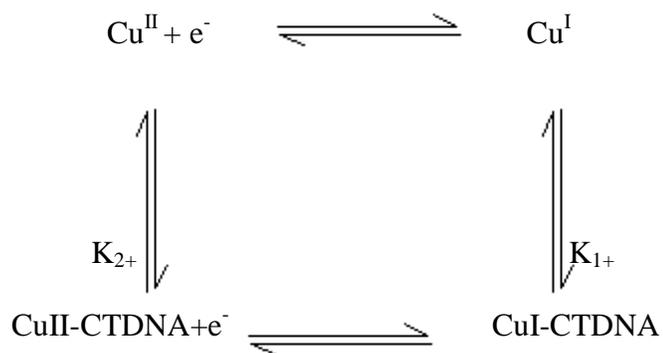


Fig. 3: Cyclic voltammogram (scan rate 0.2 Vs⁻¹, DMSO, 25 °C, pH 7.5) of

Fig. 3a: Complex 1 alone

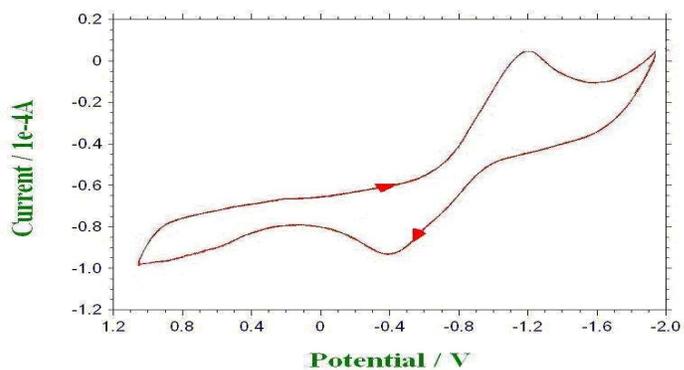


Fig. 3b: Complex 1 in presence of CT-DNA [Complex 1] 1 × 10⁻³M, [DNA] 6 × 10⁻³ M.

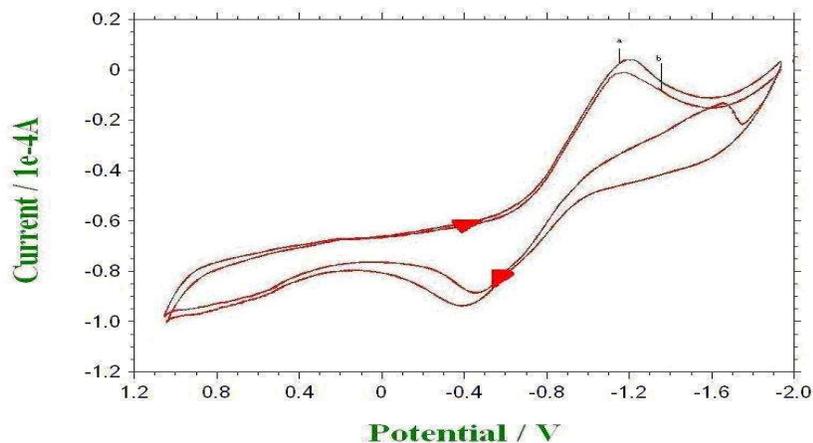


Table-6: Cyclic voltammetric data of the binuclear Schiff base Complexes in DMSO solution ^a.

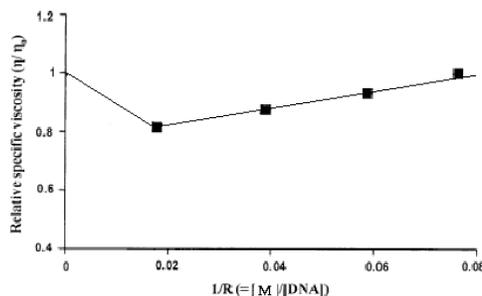
Complexes	Couple	Epc (V)	Epa (V)	ΔE_p (mv)
[Cu ₂ (L)]4ClO ₄	Cu(II)/ Cu(I)	-0.563	-0.454	0.109
[Ni ₂ (L)]4ClO ₄	Ni(II)/Ni(I)	1.60	1.85	0.25
[VO ₂ (L)]2SO ₄	VO(IV)/ VO(III)	0.61	0.75	0.14
	VO(IV)/VO(V)	-1.63	-0.52	1.11

^a Recorded in DMSO at room temperature with [t-NBu₄]ClO₄ as Supporting electrolyte, glassy carbon as working electrode. Pt wire as auxiliary electrode and Ag/AgCl as reference electrode, Scan rate 0.2 Vs⁻¹

Viscometry Studies

To obtain further support for binding modes of complexes with DNA, viscosity measurements were carried out as hydrodynamic measurements sensitive to length changes are regarded as the most critical tests of a binding model in solution in the crystallographic structural data [41]. For DNA binding of a complex, a partial or a non-classical mode of binding could bend or kink the DNA helix, reduce its effective length and concomitantly its viscosity [42, 43]. The effect of complex 1 on the viscosity of CT-DNA is shown in Fig. 4. The relative specific viscosity decreases steadily, which implies complexes bind to CT-DNA [44].

Fig. 4: Effects of increasing amount of complex 1 (•) on the relative viscosity of CT-DNA at 30 ± 0.01 °C, [DNA] = 4 x10⁻⁴ M, pH 7.2.



The changes in specific relative viscosity of DNA on addition of increasing concentrations of complex 1 are shown in Fig. 4. The decrease in relative viscosity of DNA observed for complex 1 suggests covalent binding of

complex 1 with CT-DNA, which produced bends or kinks in the DNA and thus reduced its effective length and concomitantly its viscosity. These results are consistent observed hyperchromic effect of complex 1 bound to CT-DNA covalently.

Thermal studies

TG and DTG analyses for the binuclear Schiff base metal complexes were carried out within the temperature range from ambient temperature up to 1000°C. The correlations between the different decomposition steps of the compounds with the corresponding weight losses are discussed in terms of the proposed formula of the compounds. The thermal behaviour of the complexes are summarized in Table 7 and Fig. 5. The results show good agreement with the formula suggested from the analytical data.

Table-7: Thermoanalytical data of the binuclear Schiff base complexes.

Complexes	TG range (°C)	DTGmax (°C)	Estimated (Calculated) (%)		Assignment	Metallic residue
			Mass loss	Total mass loss		
1 ^a	220-650	280, 540	81.04 (81.79)	81.04 (81.79)	Loss of four ClO ₄ , four C ₅ H ₇ N groups and aromatic (ligand) groups	CuO residue
2 ^b	240-280	270	36.92 (37.50)		Loss of four ClO ₄ groups	NiO residue
	280-420	380	30.43 (30.68)	81.63 (82.76)	Loss of four C ₅ H ₇ N groups	
	420-670	470	14.28 (14.58)		Loss of aromatic (ligand) groups	
3 ^c	180-400	390	37.01 (37.32)		Loss of four C ₅ H ₇ N groups	Decomposition is in progress
	400-450	530	17.14 (17.74)	76.15 (76.17)	Loss of aromatic (ligand) groups	
	450-750	--	22.00 (22.11)		Loss of two SO ₄ groups	

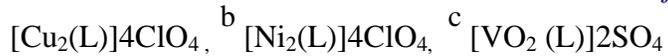


Fig. 5: Thermal analysis of binuclear Schiff base complexes

Fig. 5a: Cu-Cu complex

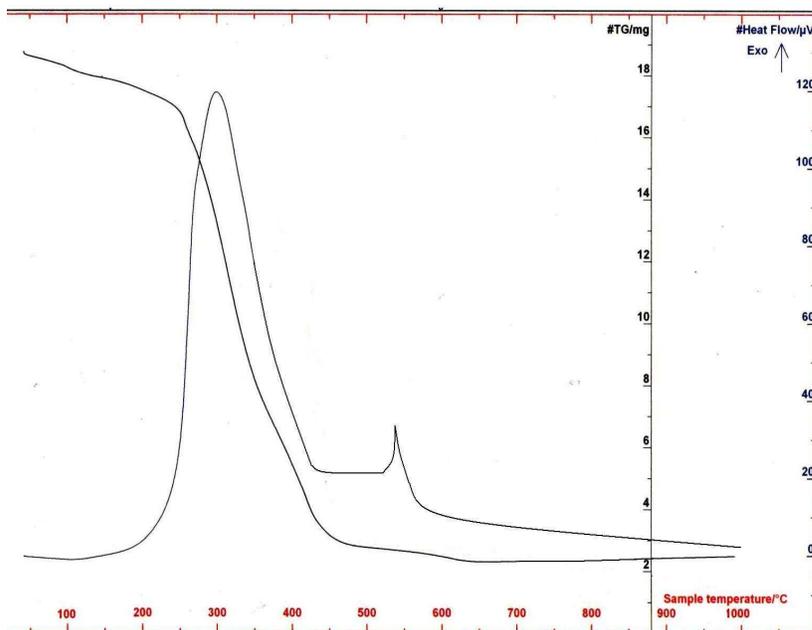


Fig. 5b: Ni-Ni complex

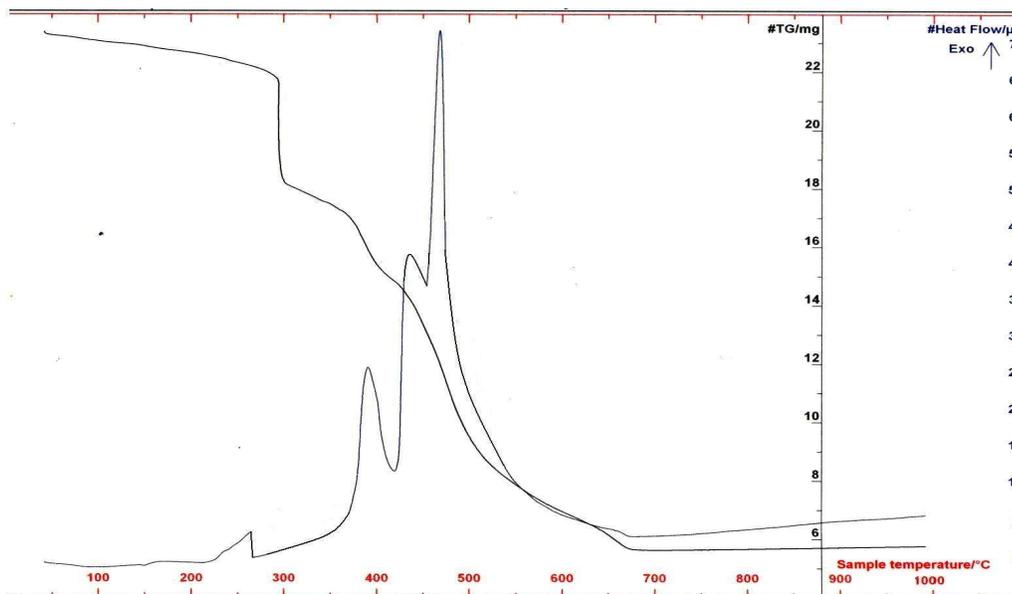
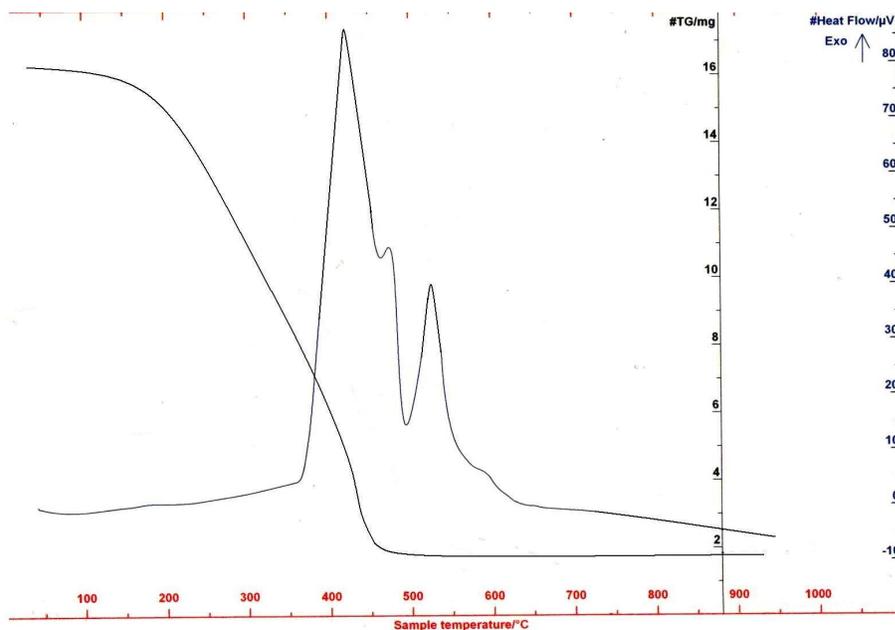


Fig. 5c: VO-VO complex.

The complex **(1)** with the molecular formula $[\text{Cu}_2\text{C}_{32}\text{H}_{38}\text{N}_4\text{O}_4]4\text{ClO}_4$ is thermally decomposed only one successive decomposition step. The estimated mass loss of 81.04% (calculated mass loss = 81.79%) within the temperature range 220-650°C may be attributed to the loss of four ClO_4 , four $\text{C}_5\text{H}_7\text{N}$ and aromatic ligand groups and CuO is suggested as a residue.

The thermal decomposition of the complex **(2)** with the molecular formula $[\text{Ni}_2\text{C}_{32}\text{H}_{38}\text{N}_4\text{O}_4]4\text{ClO}_4$ proceeds with three degradation steps. The first step occurs within the temperature range 240-280°C with an estimated mass loss 36.92% (calculated mass loss = 37.50) which is reasonably accounted for the loss of four ClO_4 . The second step occurs within the temperature range 280-420°C with an estimated mass loss 30.43% (calculated mass loss = 30.68%), which is reasonably accounted for the loss of four $\text{C}_5\text{H}_7\text{N}$ groups. The third estimated mass loss of 14.28% (calculated mass loss = 14.58%) within the temperature range 420-670°C could be attributed to the liberation of aromatic (ligand) groups. Total estimated mass loss is 81.63% (calculated mass loss = 82.76%), and NiO is suggested as a residue.

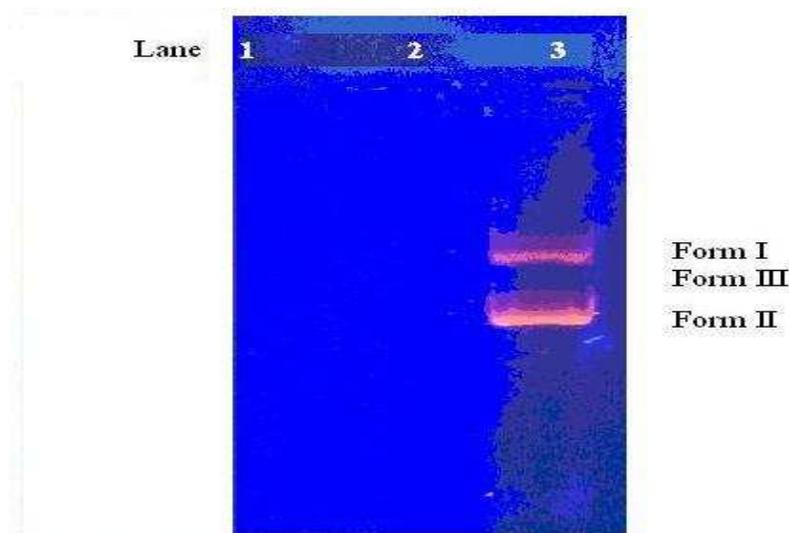
The complex **(3)** with the molecular formula $[\text{VO}_2\text{C}_{32}\text{H}_{38}\text{N}_4\text{O}_4]2\text{SO}_4$ is thermally decomposed in two decomposition steps. The first stage of decomposition within the temperature range 180-400°C with an estimated

mass loss 37.01% (calculated mass loss = 37.32%), which is attributed to the loss of four C₅H₇N groups. The second estimated mass loss of 17.14% (calculated mass loss = 17.74%) within the temperature range 400-450°C corresponding to the liberation of aromatic (ligand) groups. In the temperature range 450–750°C could be attributed to the liberation of two SO₄ groups. It is due to the decomposition of ion in progress.

Cleavage of Plasmid pUC18 DNA

DNA cleavage is controlled by relaxation of supercoiled circular form of pUC18 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between Figure.6 illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC18 DNA induced by the three binuclear complexes. Complex (3) in the presence of H₂O₂ (lane 3) at higher concentration (50μM) shows cleavage activity in which supercoiled DNA (Form-I) cleaved and supercoiled form converted to open circular form (Form-II).

Fig. 6 Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H₂O₂ and metal complexes: Lane 1, DNA + Cu(II) complex + H₂O₂; Lane 2, Ni(II) complex + H₂O₂; Lane 3, DNA + VO(IV) complex + H₂O₂;



Complexes (1) and (2) in the presence of H₂O₂ (lane 1 and 2) at higher concentration (50μM) shows more cleavage activity compared to complex (3). The supercoiled plasmid DNA was completely degraded (100 % cleavage efficiency was observed). This shows that a slight increase in the concentration over the optimal value led to extensive degradations, resulting in the disappearance of bands on agarose gel. The results revealed that the Cu(II), Ni(II) complexes have more cleavage than VO(IV) complex. 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. [48]

Antimicrobial activity

Mainly the aim of production and synthesis of antimicrobial compound is to inhibit the causal microbe without any side effects on the patients. In addition, it is worthy to stress here on the basic idea of applying any chemotherapeutic agent which depends essentially on the specific control of only one biological function and not multiple ones. Cu(II), Ni(II) and VO(IV) complexes show a remarkable biological activity against different types of Gram-positive (G+) and Gram-negative (G-) bacteria. These complexes are inhibiting Gram-positive and Gram-negative bacterial strains. The importance of this unique property of the investigated Schiff base complexes lies in the fact that, it can be applied safely in the treatment of infections and some common diseases e.g. Septicaemia, Gastroenteritis, Urinary tract infections and hospital acquired infections. The ligand and their complexes have been tested for in vitro growth inhibitory activity against gram-positive microbe *Staphylococcus aureus* and gram-negative microbe's *Klebsiella pneumonia*, *Escherichia coli* by using well-diffusion method. As the test solution concentration increases, the biological activity also increases. The minimum inhibitory concentration (MIC) values of the investigated compounds are summarized in Table 8. From the table, the observed MIC values indicate that the complexes have higher antimicrobial activity. The metal complexes Cu(II), Ni(II) and VO(IV) have higher antimicrobial activity than the ligand are shown in Figs. 7(a), 7(b) and 7(c). The increase in antimicrobial activity is due to the faster diffusion of metal complexes as a whole. The increased activity of metal chelates can be explained on the basis of the overtone concept and chelation theory. According

to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility are an important factor that controls the antimicrobial activity [49]. On chelation the ligand with the N and O donor system might have inhibited enzyme production, since enzymes which require a free hydroxy group for their activity appear to be especially susceptible to deactivation by the ions of the complexes. Mainly chelation reduces the polarity of central metal ion because of partial sharing of its positive charge. With the donor groups and possible pi electron delocalization within the whole chelate ring. This chelation increases the lipophilic nature of central metal ion which favors its permeation through lipid layers of the cell membrane [50, 51]. This increased lipophilicity enhances the penetration of complexes into the lipid membrane and blocks the metal binding sites in enzymes of microorganisms. These Cu(II), Ni(II) and VO(IV) complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms.

Fig. 7: Difference between the antimicrobial activity of binuclear ligand & metal complexes.

Fig. 7a (1) ligand ($C_{32}H_{38}N_4O_4$), (2) $[Cu_2(L)]4ClO_4$, (3) $[Ni_2(L)]4ClO_4$, (4) $[VO_2(L)]2SO_4$ [X axis –Zone of Inhibition (mm)]

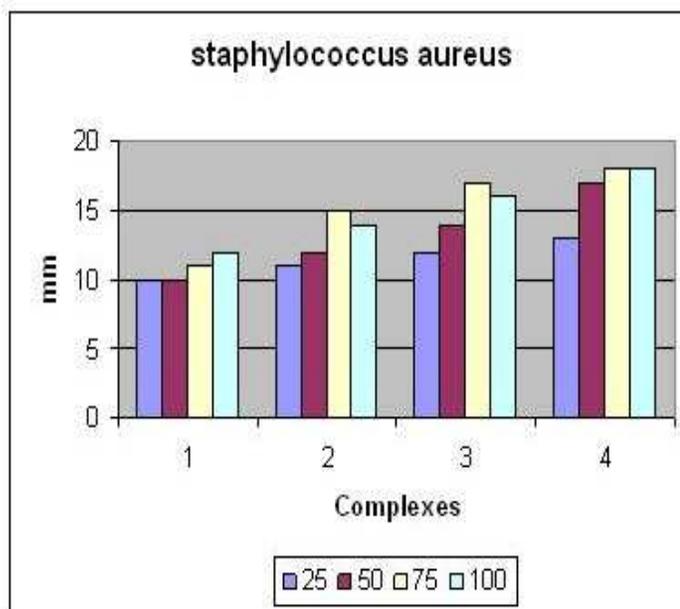


Fig. 7b Difference between the antimicrobial activity of binuclear ligand & metal complexes (1) ligand ($C_{32}H_{38}N_4O_4$), (2) $[Cu_2(L)]4ClO_4$, (3) $[Ni_2(L)]4ClO_4$, (4) $[VO_2(L)]2SO_4$. [X axis –Zone of Inhibition (mm)]

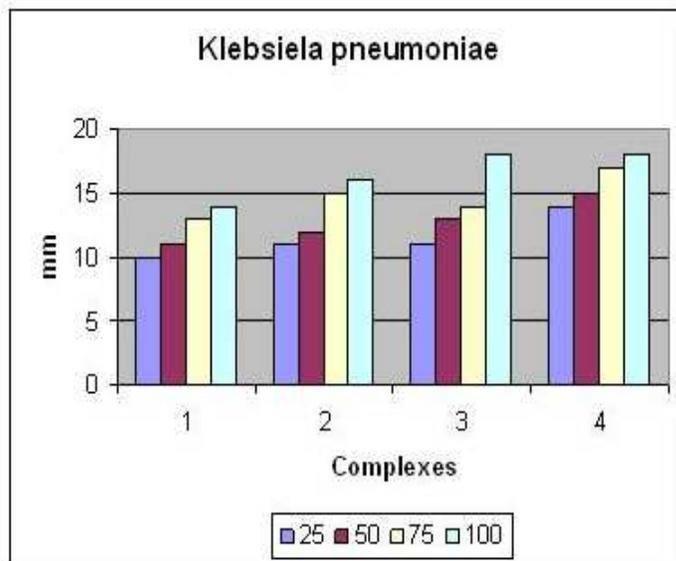


Fig. 7c Difference between the antimicrobial activity of binuclear ligand & metal complexes (1) ligand ($C_{32}H_{38}N_4O_4$), (2) $[Cu_2(L)]4ClO_4$, (3) $[Ni_2(L)]4ClO_4$, (4) $[VO_2(L)]2SO_4$. [X axis –Zone of Inhibition (mm)]

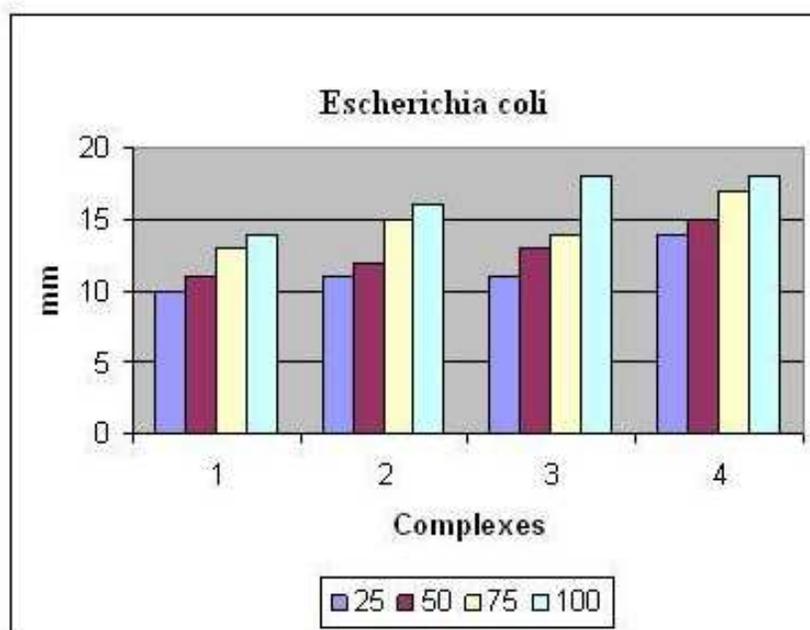


Table-8: Antibacterial activity of the ligand and binuclear Schiff base complexes.

	Klebsiella pneumoniae				Escherichia coli				Staphylococcus aureus			
	(mm)				(mm)				(mm)			
Complexes	25	50	75	100	25	50	75	100	25	50	75	100
	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)	(μ l)
(C ₃₂ H ₃₈ N ₄ O ₄)	10	11	12	13	10	11	13	14	10	12	14	16
[Cu ₂ (L)]4ClO ₄	10	12	14	17	11	12	15	16	12	13	15	16
[Ni ₂ (L)]4ClO ₄	11	15	17	18	11	13	14	18	12	14	15	19
[VO ₂ (L)]2SO ₄	12	14	16	18	14	15	17	18	11	13	17	19

Conclusion

The N₂O₂ type Schiff base ligand is synthesized from pentane-2, 4-dione and 3,3',4,4'-tetraminobiphenyl. It acts as a tetradentate ligand and forms stable complexes with transition metal ions such as Copper(II), Nickel(II), and Oxovanadium(IV). The ligand and its complexes are characterized using spectral and analytical data. The metal complexes have higher antimicrobial activity than the free ligand. The interaction of these complexes with CT-DNA was investigated by gel electrophoresis. All the transition metal complexes have higher activity than the control CT-DNA. The Cu(II), Ni(II) complexes have more activity than VO(IV) complex and the control CT-DNA.

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