Metal ion controlled synthesis of 16- and 18-membered binuclear octaazamacrocyclic complexes with Co(II), Ni(II), Cu(II) and Zn(II): a comparative spectroscopic approach to DNA binding to Cu(II) complexes

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Abstract: A series of 16- and 18-membered binuclear octaazamacrocyclic complexes, $[M_2L_1(NO_3)_4]$ and $[M_2L_2(NO_3)_4]$ (M = Co(II), Ni(II), Cu(II), Zn(II), L_1 = = 3,8,11,16-tetramethyl-1,2,4,7,9,10,12,15-octaaza-3,7,11,15-cyclohexadeca-tetraene and L_2 = 3,9,12,18-tetramethyl-1,2,4,8,10,11,13,17-octaaza-3,8,12,17- -cyclooctadecatetraene) were synthesized by metal template condensation of *N,N'*-diacetylhydrazine with 1,2-diaminoethane and 1,3-diaminopropane in methanol. The formation of macrocyclic ligand frameworks, the bonding of the macrocyclic moieties in the complexes and the overall geometry of the complexes were deduced based on the results obtained from elemental analyses as well as molar conductivity, FTIR, ^{1}H-, ^{13}C-NMR, EPR, ESI-mass, UV–Vis spectral studies and magnetic measurements. An octahedral geometry is proposed for all the complexes, while a distortion in the octahedral geometry was registered for the Cu(II) complexes. Comparative fluorescence and UV–Vis studies on the Cu(II) complexes proved a significant binding to calf thymus DNA.

Keywords: octaazamacrocycles; Cu(II), Co(II), Ni(II) and Zn(II) binuclear complexes; template condensation; DNA binding studies.

INTRODUCTION

In the last few years, a great deal of research has been aimed at designing macrocyclic compounds and studying their physico–chemical properties.[1,2] These investigations emphasized the great relevance of these systems in basic and applied chemistry. Several synthetic strategies are nowadays available for the

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preparation of well-organized molecular systems or molecular devices, which exhibit peculiar physico–chemical properties or have well defined properties.[1–4] Among the various synthetic strategies proposed, template condensation is one of the most highlighted. Metal template condensation provides selective routes towards products that are not obtainable in the absence of the metal ion.[5] The high thermodynamic stability and extreme kinetic inertness of many transition metal complexes with polyazamacrocyclic ligands are significant, as they enhance important industrial applications.[5–7] In particular, the chemistry of tetraazamacrocycles has received special attention due to their applications in a variety of catalysis, biochemical and industrial processes.[8] Hexa- and octa-azamacrocycles are known to give several mononuclear complexes, in spite of the large size of the cavity formed by the macrocyclic backbones, as well as to stabilize various anions in their protonated forms.[9–11] The synthesis of binuclear complexes has become a point of increasing interest due to their mimicry in terms of physical and chemical properties with the binuclear metal centers in enzymes.[12] A number of binuclear complexes were reported earlier due to their potential relevance in bioinorganic,[13] magneto,[14] redox[15] and coordination chemistry,[16,17] as well as in homogeneous catalysis.[18] In these systems, there is often an additional internal or external bridging group which completes the structure of the binuclear species and has the advantage of being relatively rigid, thus giving structurally well defined moieties.[19] Binuclear copper-containing proteins play an important role in biology, including dioxygen transport or activation, electron transfer, reduction of nitrogen oxides and hydrolytic consequences.[20] Hence, the design and synthesis of model compounds that mimic the physical and chemical properties of the active sites present in metalloenzymes are essential and the study of such compounds is becoming increasingly important in understanding the biological functions of bimetallic cores of enzymes.[21] In order to extend this work, herein, the synthesis and characterization is reported of novel binuclear octaazamacrocyclic complexes resulting from the template condensation of *N,N'*-diacetylhydrazine with 1,2-diaminoethane and 1,3-diaminopropane, $[M_2L_1(NO_3)_4]$ and $[M_2L_2(NO_3)_4]$, where M = Co(II), Ni(II), Cu(II), Zn(II), L_1 = 3,8,11,16-tetramethyl--1,2,4,7,9,10,12,15-octaaza-3,7,11,15-cyclohexadecatetraene and L_2 = = 3,9,12,18-tetramethyl-1,2,4,8,10,11,13,17-octaaza-3,8,12,17-cyclooctadecatetraene. Finally, the binding of the Cu(II) complexes with DNA were screened.

EXPERIMENTAL

Materials and methods

The metal salts, $Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, and $Zn(NO_3)_2 \cdot 6H_2O$, 1,2-diaminoethane, 1,3-diaminopropane (all E. Merck) and *N,N'*-diacetylhydrazine (Acros) were commercially available as pure chemicals. Methanol used as the solvent was of A.R. grade (E. Merck). Highly polymerized calf thymus DNA sodium salt (containing 7 % of Na) was purchased from Sigma. Other chemicals were of reagent grade and used without further

purification. The calf thymus DNA was dissolved to 0.50 % w/w (12.5 mM DNA/phosphate) in 0.10 M sodium phosphate buffer (pH 7.40) at 310 K for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The purity of the DNA solution was checked from the absorbance ratio A_{260}/A_{280}. Since the absorption ratio lay in the range $1.8 < A_{260}/A_{280} < < 1.9$, no further deproteinization of the DNA was required. Stock solutions of the complexes $[Cu_2L_1(NO_3)_4]$ and $[Cu_2L_2(NO_3)_4]$, synthesized as described below (abbreviated with **1c** and **2c**) (c = 5 mg/ml) were also prepared.

Synthesis of tetranitrato(3,8,11,16-tetramethyl-1,2,4,7,9,10,12,15-octaaza-3,7,11,15-cyc-lohexadecatetraene)bimetal(II) type of complexes

A methanolic solution (≈ 25 ml) of 1,2-diaminoethane (0.54 ml, 8.0 mmol) and *N,N'*-diacetylhydrazine (0.93 g, 8.0 mmol) in methanol (≈ 25 ml) were simultaneously added dropwise to stirred methanolic solution (≈ 25 ml) of metal salt (8.0 mmol). The resultant mixture was stirred for several hours leading to isolation of the solid product, which was then filtered off, washed several times with methanol and dried under vacuum to give the complex $[M_2L_1(NO_3)_4]$ (M = Co(II) (**1a**), Ni(II) (**1b**), Cu(II) (**1c**) and Zn(II) (**1d**)).

Synthesis of tetranitrato(3,9,12,18-tetramethyl-1,2,4,8,10,11,13,17-octaaza-3,8,12,17-cy-clooctadecatetraene)bimetal(II)type of complexes

The procedure was similar to the one mentioned above, except 1,3-diaminopropane (0.67 ml, 8.0 mmol) was used instead of 1,2-diaminoethane, whereby the complexes $[M_2L_2(NO_3)_4]$ (M = Co(II) (**2a**), Ni(II) (**2b**), Cu(II) (**2c**) and Zn(II) (**2d**)) were obtained.

Binding analysis of complexes 1c and 2c

To elaborate the fluorescence quenching mechanism, the Stern-Volmer equation was used for data analysis:[22]

$$F_0/F = 1 + K_{SV}[Q] \tag{1}$$

where F_0 and F are the steady-state fluorescence intensities in the absence and presence of quencher, respectively. K_{SV} is the Stern–Volmer quenching constant and [Q] is the concentration of quencher (DNA). The values of K_{SV} for complexes **1c** and **2c** were found to be of the order of 10^4. The linearity of the F_0/F *vs.* [Q] (Stern–Volmer) plots for the DNA–**1c** and DNA–**2c** complexes (Fig. 1) indicates that the quenching may be static or dynamic, since the
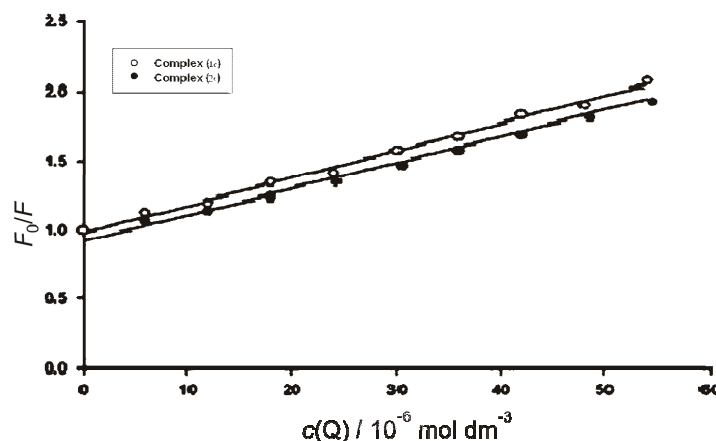

Fig. 1. Stern–Volmer plot for the binding of complex **1c** and **2c** with DNA at 298 K, pH 7.4.

characteristic Stern–Volmer plot of combined quenching (both static and dynamic) has an upward curvature. When ligand molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation:[23]

$$\log [(F_0 - F)/F] = \log K + n\log [Q] \qquad (2)$$

where K and n are the binding constant and the number of binding sites, respectively. Thus, a plot of $\log (F_0 - F)/F$ *vs.* $\log [Q]$ can be used to determine K as well as n.

Physical measurements

Elemental analyses were obtained from C. D. R. I. Lucknow, India. The IR spectra (4000–200 cm^{-1}) of the complexes were recorded as CsI/KBr discs on a Perkin-Elmer-621 spectrophotometer. The ESI-mass spectra were obtained by electrospray ionization on a Micromass Quattro II triple quadrupole mass spectrometer from C.D.R.I. Lucknow. The ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 using a Jeol FT NMR AL-300 MHz spectrometer with Me_4Si from G. N. D. U. Amritsar India as the internal standard. The electronic spectra of the complexes in DMSO were recorded on Pye-Unicam 8800 spectrophotometer. The magnetic susceptibility measurements were performed using a Faraday balance at 25 °C. The data were corrected for diamagnetic susceptibilities using Pascal's constants. The EPR spectra of the Cu(II) complexes were recorded as powder samples at room temperature on an E-4 spectrometer using DPPH as the *g*-marker. The molar conductivity data for 10^{-3} M solution in DMSO were recorded on a Systronic type 302 conductivity bridge thermostated at 25.00±0.05 °C. The contents of metals were determined volumetrically.[24] Fluorescence measurements were performed on a spectrofluorimeter Model RF-5301PC (Shimadzu, Japan) equipped with a 150W Xenon lamp using a slit width of 5 nm. A 1.00 cm quartz cell was used for the measurements. For the determination of binding parameters, 30 μM of complex solution was taken in a quartz cell and increasing amounts of CT DNA solution were titrated. Fluorescence spectra were recorded at 310 K in the range of 740–880 nm with excitation at 280 (λ_{em} was 770 nm). The UV measurements of calf thymus DNA were recorded on a Shimadzu double beam spectrophotometer model UV 1700 using a 1 cm path length cuvette. Absorbance values of DNA in the absence and presence of complex were measured in the range of 220–300 nm. DNA concentration was fixed at 0.10 mM, while the compound was added in increasing concentrations.

RESULTS AND DISCUSSION

The metal ion controlled reaction of the 1,2-diaminoethane and 1,3-diaminopropane with *N,N'*-diacetylhydrazine in 1:1:1 molar ratio resulted in the formation of new series of 16- and 18-membered binuclear Schiff-base macrocyclic complexes of the types $[M_2L_1(NO_3)_4]$ and $[M_2L_2(NO_3)_4]$, where M = Co(II), Ni(II), Cu(II) and Zn(II) (Scheme 1). The resulting complexes were obtained as colored solids in moderate yields (54–65 %). All the complexes were soluble in polar solvents and were stable at room temperature. The purity of the complexes was checked by TLC on a silica gel coated plate using EtOAc–MeOH (6:4 v/v) as the eluent. The elemental analyses (Table I) agree well with the proposed stoichiometry of the binuclear octaazamacrocyclic complexes. The positions of the molecular ion peaks in the mass spectra were consistent with the empirical mole-

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cular formulae (Table I). The molar conductivities (Table I) of all the complexes in DMSO corresponded to a non-electrolytic[25] nature of these complexes.
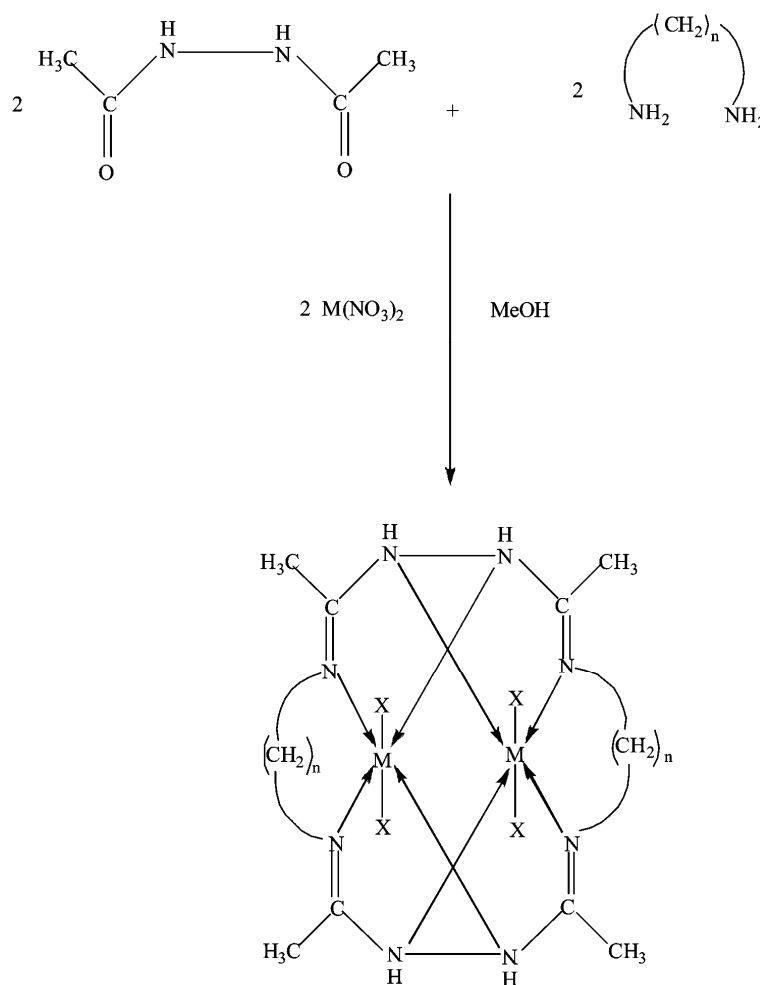
Scheme 1. M = Co(II), Ni(II), Cu(II) and Zn(II), X = NO_3^-, $n = 2$ for the complex $[M_2L_1(NO_3)_4]$ and $n = 3$ for the complex $[M_2L_2(NO_3)_4]$.

Elemental analyses

Anal. Calcd. for $[Co_2L_1(NO_3)_4]$ ($C_{12}H_{24}Co_2N_{12}O_{12}$): C, 22.22; H, 3.58; N, 25.82; Co, 18.00. Found: C, 22.30; H, 3.74; N, 26.00, Co, 18.23. Anal. Calcd. for $[Co_2L_2(NO_3)_4]$ ($C_{14}H_{28}Co_2N_{12}O_{12}$): C, 24.66; H, 4.09; C, 24.77; Co, 17.38. Found: C, 24.93; H, 4.18; N, 24.92; Co, 17.47. Anal. Calcd. for $[Ni_2L_1(NO_3)_4]$ ($C_{12}H_{24}N_{12}Ni_2O_{12}$): C, 22.0; H, 3.62; N, 26.00; Ni, 18.14. Found: C, 22.31; H, 3.74; N, 26.02; Ni, 18.17. Anal. Calcd. for $[Ni_2L_2(NO_3)_4]$ ($C_{14}H_{28}N_{12}Ni_2O_{12}$):

C, 24.73; H, 4.15; N, 24.52; Ni, 17.22. Found: C, 24.95; H, 4.18; N, 24.94; Ni, 17.42. Anal. Calcd. for $[Cu_2L_1(NO_3)_4]$ ($C_{12}H_{24}Cu_2N_{12}O_{12}$): C, 21.82; H, 3.37; N, 25.32; Cu, 19.00. Found: C, 21.98; H, 3.69; N, 25.64; Cu, 19.38. Anal. Calcd. for $[Cu_2L_2(NO_3)_4]$ ($C_{14}H_{28}Cu_2N_{12}O_{12}$): C, 24.52; H, 4.10; N, 24.32; Cu, 18.28. Found: C, 24.60; H, 4.12; N, 24.58; Cu, 18.59. Anal. Calcd. for $[Zn_2L_1(NO_3)_4]$ ($C_{12}H_{24}N_{12}O_{12}Zn_2$): C, 21.73; H, 3.54; N, 25.21; Zn, 19.62. Found: C, 21.86; H, 3.66; N, 25.49; Zn, 19.83. Anal. Calcd. for $[Zn_2L_2(NO_3)_4]$ ($C_{14}H_{28}N_{12}O_{12}Zn_2$): C, 24.30; H, 4.01; N, 24.39; Zn, 19.00. Found: C, 24.46; H, 4.10; N, 24.45; Zn, 19.02.

TABLE I. Elemental analyses, *m/z* values, color, yield, molar conductance and melting point values of the prepared complexes

Compound	*m/z* Found (Calcd.)	Color	Yield, %	Molar conductance S mol^{-1} cm^2	M.p. °C
$[Co_2L_1(NO_3)_4]$ (**1a**) $C_{12}H_{24}N_{12}Co_2O_{12}$	646.20 (646.25)	Brown	58	13	>300 °C
$[Co_2L_2(NO_3)_4]$ (**2a**) $C_{14}H_{28}N_{12}Co_2O_{12}$	674.00 (674.31)	Dark brown	65	17	>300 °C
$[Ni_2L_1(NO_3)_4]$ (**1b**) $C_{12}H_{24}N_{12}Ni_2O_{12}$	645.52 (645.77)	Violet	55	19	>300 °C
$[Ni_2L_2(NO_3)_4]$ (**2b**) $C_{14}H_{28}N_{12}Ni_2O_{12}$	673.80 (673.82)	Purple	63	21	>300 °C
$[Cu_2L_1(NO_3)_4]$ (**1c**) $C_{12}H_{24}N_{12}Cu_2O_{12}$	655.25 (655.48)	Blue	62	23	>300 °C
$[Cu_2L_2(NO_3)_4]$ (**2c**) $C_{14}H_{28}N_{12}Cu_2O_{12}$	683.50 (683.54)	Dark blue	65	18	>300 °C
$[Zn_2L_1(NO_3)_4]$ (**1d**) $C_{12}H_{24}N_{12}Zn_2O_{12}$	659.10 (659.15)	Colorless	60	24	>300 °C
$[Zn_2L_2(NO_3)_4]$ (**2d**) $C_{14}H_{28}N_{12}Zn_2O_{12}$	687.09 (687.20)	Colorless	54	20	>300 °C

FTIR spectra

The IR spectra of the binuclear complexes of the type $[M_2L_1(NO_3)_4]$ and $[M_2L_2(NO_3)_4]$ exhibited characteristic bands of the expected functional groups and relevant data are given in Table II. The formation of the macrocyclic complexes was confirmed by the appearance of a ν(C=N) band[26] in the region 1600–1620 cm^{-1} and the absence of the ν(NH$_2$) bands at $\approx$ 3400 cm^{-1}, indicating that a Schiff base condensation between the carbonyl group of diacetylhydrazine and the amino group of the alkylamine had occurred. This fact is further supported by the presence of a medium intensity ν(M–N) band, observed in the 382–400 cm^{-1} region.[26] In addition to the above, a medium intensity band appeared in the region 3230–3250 cm^{-1}, which may be assigned to the ν(N–H) stretching vibration of the secondary amine of the condensed diacetylhydrazine moiety.[27] The absorption band appearing in the region 2870–2920 cm^{-1} may be due to the ν(C–H)

stretching vibration. Moreover, the strong band at around 950–973 cm^{-1} may be ascribed to the ν(N–N) stretching mode of the condensed diacetylhydrazine moiety.[28] The coordination of the nitrato group with the metal was ascertained by the bands in the 230–245 cm^{-1} region, which may reasonably be assigned to the ν(M–O) of the (O–NO$_2$) group. The spectra of the metal complexes gave additional bands at around 1235–1260, 1028–1060 and 855–870 cm^{-1}, which are consistent with the monodentate coordination of nitrate anions.[29]

TABLE II. IR spectral data (cm^{-1}) of the complexes

Compound	ν(C=N)	ν(N–N)	ν(N–H)	ν(M–N)	ν(M–O)
[Co$_2$L$_1$(NO$_3$)$_4$]	1608(*s*)	952(*s*)	3236(*m*)	382(*m*)	242(*m*)
[Co$_2$L$_2$(NO$_3$)$_4$]	1600(*s*)	970(*s*)	3248(*m*)	394(*m*)	230(*m*)
[Ni$_2$L$_1$(NO$_3$)$_4$]	1617(*s*)	962(*s*)	3233(*m*)	395(*m*)	245(*m*)
[Ni$_2$L$_2$(NO$_3$)$_4$]	1603(*s*)	955(*s*)	3245(*m*)	390(*m*)	233(*m*)
[Cu$_2$L$_1$(NO$_3$)$_4$]	1619(*s*)	966(*s*)	3230(*m*)	386(*m*)	238(*m*)
[Cu$_2$L$_2$(NO$_3$)$_4$]	1610(*s*)	950(*s*)	3250(*m*)	388(*m*)	240(*m*)
[Zn$_2$L$_1$(NO$_3$)$_4$]	1615(*s*)	958(*s*)	3240(*m*)	398(*m*)	235(*m*)
[Zn$_2$L$_2$(NO$_3$)$_4$]	1620(*s*)	973(*s*)	3242(*m*)	400(*m*)	244(*m*)

^{1}H-NMR spectra

The ^{1}H-NMR spectra of the **1d** and **2d** complexes exhibited resonance peaks at δ 6.20 and 6.23 ppm for secondary amino protons (4H, –C–NH–N–) and δ 2.09 and 2.15 ppm for imine methyl[30] protons (12H, CH$_3$C=N–) of the condensed diacetylhydrazine moiety. A singlet at δ 3.16 and 3.25 ppm for **1d** and **2d**, respectively, may reasonably be assigned to methylene protons (8H, –N–CH$_2$–C–) of the condensed amino moiety. While another singlet observed at δ 1.98 ppm may reasonably be assigned to the middle methylene protons (4H, –C–CH$_2$–C–) of the propane moiety in **2d**.[31]

^{13}C-NMR spectra

The ^{13}C-NMR spectra for Zn(II) complexes revealed the presence of the imine moiety (>C=N–) at 158 and 160 ppm in both complexes.[32] The chemical shifts of the (>N–CH$_2$–) carbons appear at 45 and 43 ppm for 1,2-diaminoethane and 1,3-diaminopropane, respectively.[30] Moreover, the resonance peaks observed at 22 and 23 ppm correspond to the four methyl carbon adjacent to imine group.[30] Another signal observed at 33 ppm was assigned to the middle carbon atom (–CH$_2$–) of the 1,3-diaminopropane moiety.[32]

EPR spectra

The EPR spectra of the complexes **1c** and **2c** were recorded at room temperature and their $g_{\parallel}$ and $g_{\perp}$ values were calculated (Table III). Both the complexes exhibited a similar single absorption band (Figs. 2 and 3). The absence of hyperfine lines in the spectra of these complexes may be due to the strong dipolar and ex-

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change interactions between the Cu(II) ions in the unit cell. The calculated $g_\parallel$ values of 2.112 and 2.147 and the $g_\perp$ values of 2.071 and 2.047 for **1c** and **2c**, respectively, support the fact that $^2B_{1g}$ is the ground state, having an unpaired electron in the $d_{x^2-y^2}$ orbital of the Cu(II) ion. Both the complexes show $g_\parallel < 2.3$, indicating that the present complexes exhibit appreciable covalent nature.[33] The observed $g_\parallel < 2.0023$ for the complexes show that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of the Cu(II) ion, which is characteristic of the axial symmetry. Tetragonally elongated geometry[34] is thus confirmed for the aforesaid complexes.

Table III. Magnetic moment values, electronic spectral data with their assignments and EPR spectral parameters of the complexes

| Compound	μ_{eff} / μ_B	Band position cm^{-1}	Assignments	EPR parameters		
				$g_\parallel$	$g_\perp$	G
$[Co_2L_1(NO_3)_4]$	4.60	9000	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			
		17000	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$			
		21850	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			
$[Co_2L_2(NO_3)_4]$	4.57	9090	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$			
		16750	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$			
		21600	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$			
$[Ni_2L_1(NO_3)_4]$	3.34	11400	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$			
		17500	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$			
$[Ni_2L_1(NO_3)_4]$	3.40	11200	$^5A_{2g}(F) \rightarrow {}^5T_{2g}(F)$			
		17600	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$			
$[Cu_2L_1(NO_3)_4]$	1.90	13000	$^2B_{1g} \rightarrow {}^2A_{1g}$	2.112	2.071	1.577
		17000	$^2B_{1g} \rightarrow {}^2E_g$			
$[Cu_2L_2(NO_3)_4]$	1.87	13750	$^2B_{1g} \rightarrow {}^2A_{1g}$	2.147	2.047	3.127
		16650	$^2B_{1g} \rightarrow {}^2E_g$			
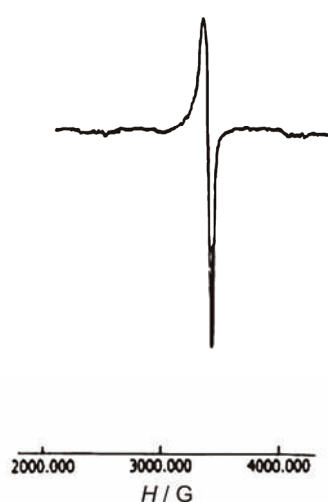
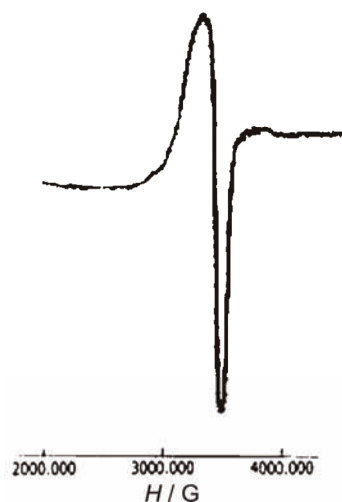Fig. 2. X-band EPR spectrum of the **1c** complex at room temperature.

Fig. 3. X-band EPR spectrum of the **2c** complex at room temperature.

The G values are related by the expression $G = (g_\parallel - 2) / (g_\perp - 2)$, and lie in the range 1.577 and 3.127 for the complexes **1c** and **2c**, indicating a significant exchange interaction[35] among the Cu(II) ions in these complexes, as the G values were less than 4.

Electronic spectra and magnetic susceptibility data

The electronic spectra of the binuclear Co(II) complexes (Table III) showed three bands in the range 9,000, 17,000 and 21,850 cm^{-1} for **1a** and 9,090, 16,750 and 21,600 cm^{-1} for **2a** complexes, attributed to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively, consistent with an octahedral geometry[36] around the Co(II) ion. The magnetic moment values of 4.60 μ_B for the **1a** and 4.57 μ_B for the **2a** complex (Table III) correspond to high spin Co(II) in an octahedral environment. The relatively higher values of the observed magnetic moments than that of the spin only moment (3.89 μ_B) may be attributed in terms of the orbital contribution[36] generally observed for Co(II) compounds.

The Ni(II) ion complexes exhibit two absorption bands in the region 11,400 and 17,500 cm^{-1} for the **1b**, and 11,200 and 17,600 cm^{-1} for the **2b** complex, corresponding to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transitions, respectively, suggesting an octahedral geometry.[37] Further confirmation regarding the octahedral environment around the Ni(II) ion was deduced from the magnetic moment values of 3.34 μ_B and 3.40 μ_B for the **1b** and **2b** complexes, respectively (Table III).

The electronic spectra of the hexacoordinated Cu(II) complexes showed three spin allowed transitions in the visible and near IR regions. These bands were assigned as $^2B_{1g} \rightarrow {}^2A_{1g}$ ($d_{x^2-y^2} - d_{z^2}$), $^2B_{1g} \rightarrow {}^2B_{2g}$ ($d_{x^2-y^2} - d_{xy}$) and $^2B_{1g} \rightarrow {}^2E_g$ ($d_{x^2-y^2} - d_{xz}, d_{yz}$) transitions in the increasing order of their energies. The energy level sequence will depend on the amount of tetragonal distortion due to the ligand field and the Jahn–Teller effect.[38,39] The electronic spectra of the complexes reported here show two characteristic bands in the range 13000 and 17000 cm^{-1} for the **1c** and 13750 and 16650 cm^{-1} for the **2c** complex. These may reasonably be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively. A band corresponding to the $^2B_{1g} \rightarrow {}^2B_{2g}$ transition was not observed as a separate band, which may be due to tetragonal distortion.[39] The observed magnetic moments of 1.90 μ_B for the **1c** and 1.87 μ_B for the **2c** complex further supplement the electronic spectral findings (Table III).

Fluorescence measurements

Interactions of DNA with complexes 1c and 2c. Fluorescence spectroscopy provides insight into changes occurring in the DNA microenvironment on addition of the complexes. The interaction of the compounds with calf thymus DNA was studied by monitoring the changes in the intrinsic fluorescence of these com-

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pounds at varying DNA concentrations. Representative fluorescence emission spectra of the compounds upon excitation at 290 nm are shown in Figs. 4A and 4B. The addition of DNA caused a gradual decrease in the fluorescence emission intensity of both compounds, with a conspicuous change in the emission spectra. The spectra illustrate that higher excesses of DNA led to more effective quenching of the fluorescence of the fluorophore molecule. The quenching of fluorescence clearly indicates that the binding of the DNA to complexes **1c** and **2c** changed the microenvironment of the fluorophore residue. The shift in emission peak of the synthesized molecules further depicts an effective interaction at higher DNA concentration, which was more prominent in the case of **1c**. The reduction in the intrinsic fluorescence upon interaction with DNA could be due to masking or burial of the fluorophore between the stacked bases within the helix and/or surface binding at the reactive nucleophilic sites on the heterocyclic nitrogenous bases of DNA.
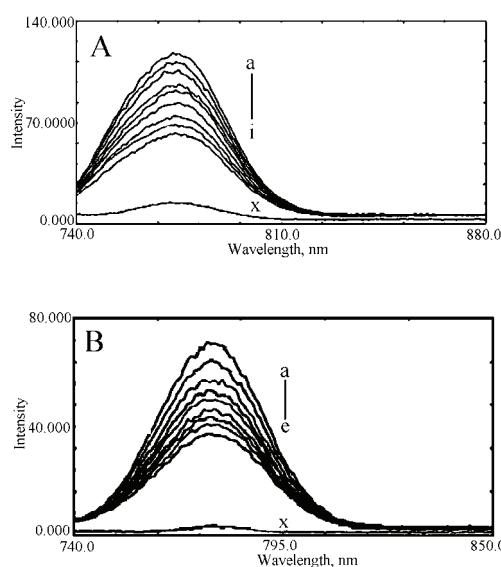

Fig. 4. Fluorescence emission spectra of the complexes in the absence and presence of increasing amounts of DNA. Excitation 290 nm. Fixed concentration of complex (*i.e.*, 4 µM) was titrated in each titration. A concentration of 4 µM DNA (x) was used for DNA alone. Spectra (A) and (B) represent complex **1c** and **2c**, respectively.

The binding parameters for complex **1c** and **2c** were found to be $K = (8.21\pm0.21)\times10^4$ mol^{-1} dm^3, $n = 1.1$ and $K = (0.74\pm0.11)\times10^4$ mol^{-1} dm^3, $n = 0.92$, respectively. Pronounced bathochromism was observed with complex **1c**, which was negligible with complex **2c**. The results suggest that the compounds have varying degrees of affinity toward the DNA molecule. This differential binding of **1c** and **2c** is attributed in terms of different molecular structures around the Cu(II) ion.

Absorption spectroscopy. UV–Vis absorption studies were performed to further ascertain the DNA–complex **1c** and **2c** interaction. The UV absorbance show-

ed an increase with increasing complex **1c**/**2c** concentrations (Fig. 5A). Since complexes **1c** and **2c** do not show any peak in this region (Fig. 5B), the increase in the DNA absorbance is indicative of interaction between DNA and the complexes. Both complexes (**1c** and **2c**) exhibited hyperchromism but of varying degrees. Hyperchromism means the breakage of the secondary structure of DNA. Hence, it is primarily speculated that the complexes interact with the secondary structure of calf thymus DNA, resulting in its breakage and perturbation. After interaction with the base pairs of DNA, the $\pi \rightarrow \pi^*$ orbital of the bound ligand can couple with the π orbital of the base pairs, due to the decrease in $\pi \rightarrow \pi^*$ transition energy, which results in a bathochromic shift.[40] The prominent shift in the spectra of **1c** suggests more interference of the orbital by the complex **1c** molecule. The above changes are indicative of conformational alteration of DNA but of varied extent.
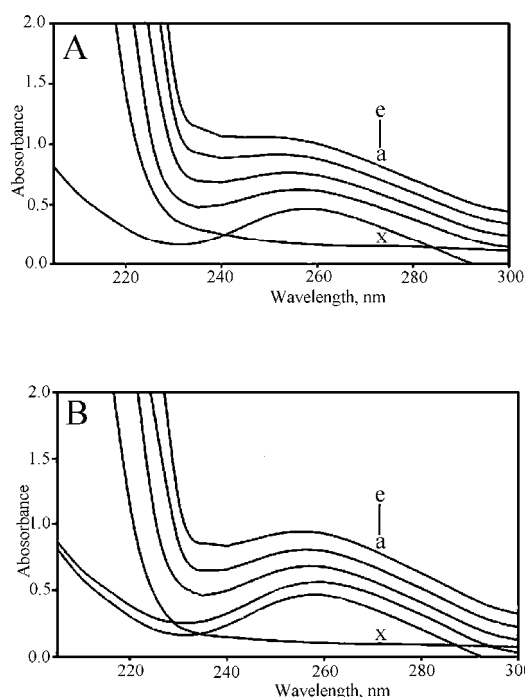

Fig. 5. Absorbance spectra of DNA and DNA–**1c** (A)/**2c** (B) system. The DNA concentration was 0.10 mM (a); complex concentration for DNA–**1c** and **2c** complex systems was a) 10, b) 20, c) 30 and d) 40 µM.

CONCLUSIONS

A novel series of 16- and 18-membered Schiff-base binuclear N_8-macrocyclic complexes were synthesized and characterized by spectral and elemental analyses. The IR spectra of the complexes showed the absence of bands corresponding to the $\nu(C=O)$ and $\nu(NH_2)$ group of diacetylhydrazine and alkylamine moieties, respectively, and the appearance of a new well-defined band corresponding

to the ν(C=N) stretching vibration. This suggests the formation of the macrocyclic framework, which was further substantiated by the ^{1}H- and ^{13}C-NMR spectra. Confirmation regarding the stoichiometry, nature and overall geometry around the metal ion was further deduced from the electronic, ESI-mass, EPR spectra, magnetic moment values and conductivity measurements. Fluorescence and absorption measurements demonstrated a considerable interaction between the Cu(II) complexes **1c** and **2c** and CT DNA.

И З В О Д

СИНТЕЗА 16- И 18-ОЧЛАНИХ БИНУКЛЕАРНИХ ОКТААЗАМАКРОЦИКЛИЧНИХ КОМПЛЕКСА Co(II), Ni(II), Cu(II) И Zn(II) КОНТРОЛИСАНА МЕТАЛНИМ ЈОНИМА: УПОРЕДНИ СПЕКТРОСКОПСКИ ПРИКАЗ ВЕЗИВАЊА DNA ЗА Cu(II) КОМПЛЕКСЕ

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Добијена је серија 16- и 18-очланих бинуклеарних октаазамакроцикличних комплекса, $[M_2L_1(NO_3)_4]$ и $[M_2L_2(NO_3)_4]$ (M = Co(II), Ni(II), Cu(II), Zn(II), L_1 = 3,8,11,16-тетраметил--1,2,4,7,9,10,12,15-октааза-3,7,11,15-циклохексадекатетрен и L_2 = 3,9,12,18-тетраметил-1,2,4,8,10,11,13,17-октааза-3,8,12,17-циклооктадекатетраен) металном темплатном кондензацијом *N,N*'-диацетилхидразина са 1,2-диаминоетаном и 1,3-диаминопропаном у метанолу. Грађење макроцикличне лигандне структуре, везивање макроцикличне средине комплекса у општу геометрију изведено је на основу резултата елементалне анализе, моларне проводљивости, FT-IR, ^{1}H, ^{13}C-NMR, EPR, ESI-масеног, UV–Vis спектралног проучавања и магнетних мерења. Октаедарска геометрија је предложена за све комплексе, док је дисторгована октаедарска геометрија примећена код Cu(II) комплекса. Упоредна флуоресцентна и UV–Vis проучавања Cu(II) комплекса доказала су значајно везивање за DNA телећег тимуса.

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