Turk J Chem
36 (2012) , 717 – 733.

doi:10.3906/kim-1110-3

Synthesis and characterization of bis(azine) ligands and metal complexes: DNA-interaction and extraction properties for metals and dichromate anions

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Received: 03.10.2011

Two new bis(bidentate) azine ligands were prepared by linking (1*Z*,1′ *Z*)-1,1′-{butane-1,4-diylbis[oxybenzene-4,1-diyl(1*Z*)eth-1-yl-1-ylidene]}dihydrazine to salicylaldehyde and pyridine-2-carbaldehyde, 1,4-bis{4-{(1*E*)-1-((2*E*)-(2-hydroxybenzene–2-ylmethy-lidene)hydrazonylidene)ethyl}phenoxy}butane (H_2L^1), and 1,4-bis{4-{(1*E*)-1-((2*E*)-(pyridine–2-ylmethylidene)hydrazonylidene)ethyl}phenoxy}butane (L^2), respectively. Two kinds of copper(II) and nickel(II) complexes with different ligands were prepared. Reaction of H_2L^1 with Cu(II) and Ni(II) acetate with 1:1 molar ratio gave double stranded binuclear bis(azine) complexes with stoichiometry $[M(L^1)]_2$ containing $\{M^{II}N_2O_2\}$ centers. On the other hand, 1:1 molar ratio reaction of Cu(II) and Ni(II) chloride with L^2 yielded mononuclear metal complexes with general stoichiometry $[M(L^2)Cl_2]$ containing $\{M^{II}N_2N_2\}$ centers. The structures of both bis(azine) ligands and complexes were identified by elemental analysis, infrared spectra, UV–Vis electronic absorption spectra, magnetic susceptibility measurements, and TGA. DNA binding and DNA cleavage activities of the mononuclear complexes of L^2, $[M(L^2)Cl_2]$ were examined by using UV-Vis titration and agarose gel electrophoresis, respectively. The results indicate that mononuclear complexes, especially $[Cu(L_2)Cl_2]$, bind significantly calf thymus DNA and cleaves pBR322 DNA. Furthermore, the complexing properties of the bis-azine ligands toward selected transition metal cations and dichromate anions were also reported. It was found that bis(azine) ligands have high extraction ability towards dichromate anions.

Key Words: Bis(azine), complexes, DNA-interaction, extraction, heavy metals, dichromate anions

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Introduction

Azines (R_2C=N-N=CR_2) are 2-3-diaza analogues of 1,3-butadine. The chemical properties of azines have been extensively investigated in the past decades because of their interesting physical and spectral properties.[1,2] Azines are also potential ligands owing to their having 2 imine groups side by side. Therefore, they have recently been used as ligands in coordination chemistry.[2–7] The azines can be considered as models of some biological systems.[8,9] The stereochemistry and the stereoelectronics of some substituted acetophenone azines have been recently studied[10,11] since these compounds are considered as nonlinear optical materials.

The interaction of coordination compounds with DNA has been intensively studied in the past decades.[12–14] Coordination compounds are suitable candidates as DNA secondary structure probes, photocleavers, and antitumor drugs.[15,16] In particular, Schiff base complexes have attracted much attention due to their significance in the development of new therapeutic agents and novel nucleic acid structural probes.[15,16] Artificial metallonucleases can be potentially used in gene regulation, mapping of protein and DNA interactions, probing of DNA specific structures, and in cancer therapy.[12–17] Therefore, the development of artificial nucleases is very important in both medicinal and biotechnology fields.

Many heavy metals such as copper, mercury, chromium, lead, nickel, and cadmium are released in wastewaters through the industrial process.[18–20] Chromium and its compounds are used in diverse industrial areas such as plating, leather tanning, dye and photographic industries where large amounts of toxic pollutants are released into the environment.[19–20] Chromium can exist in several oxidation states like Cr(III) and Cr(VI). Cr(III) is reported to be biologically essential, while chromium(VI) can be toxic since it can diffuse as $Cr_2O_7^{2-}$ or $HCr_2O_7^-$ through cell membranes and oxidize biological molecules.[21] Therefore heavy metal pollution is spreading throughout the world with the expansion of industrial activities. Many methods for the removal of heavy metals from wastewaters such as chemical precipitation, membrane filtration, coagulation, complexing, solvent extraction, ion exchange and adsorption are used. Among them, the most widespread procedure is solvent extraction. Therefore, development of an efficient extractant is very important.

Although many works have been devoted to study the structure of azines,[2–5] less research has been attempted concerning the complexes of the azine compounds and their bioinorganic relevance.[6–9] Therefore, herein we described the synthesis and characterization of new bis(azine)s and their mono- and binuclear Cu(II) and Ni(II) complexes. DNA binding and cleavage activities of these complexes were discussed. The extraction property of this azine toward dichromate anion and some heavy metals was also studied by solvent extraction.

Experimental

Materials

All chemicals used were of the analytical reagent grade. $CuCl_2 \cdot 2H_2O$, $Cu(AcO)_2 \cdot H_2O$, $NiCl_2 \cdot 6H_2O$, $Ni(AcO)_2 \cdot 4H_2O$, 4-hydroxyacetophenone, 1,4-dibromopropan, salicylaldehyde, pyridine-2-carbaldehyde, $Na_2Cr_2O_7$, K_2CO_3, acetone, and hydrazine monohydrate were purchased from Fluka and Sigma-Aldrich and used without further purification. Calf thymus DNA (CT-DNA) was purchased from Sigma-Aldrich. pBR322 DNA was purchased from Fermantas.

Physical measurements

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-D6 with TMS as the internal standard. IR spectra were recorded on a Perkin-Elmer 1605 FTIR spectrometer as KBr pellets. The electronic spectra of the ligands and complexes were recorded on a UV-1601 Shimadzu spectrophotometer. Carbon, hydrogen, and nitrogen analyses were carried out on a LECO 932 CHNS analyzer and metal contents were determined by atomic absorption spectroscopy using a DV 2000 Perkin Elber ICP-AES. Room temperature magnetic susceptibility measurements were carried out on powdered samples using a Sherwood Scientific MK1 Model Gouy Magnetic Susceptibility Balance. Melting points were determined on an Electrothermal IA 9100 digital melting point apparatus. The thermogravimetric analysis was carried out in dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of 20 °C min^{-1} using a Perkin Elmer Pyres 1 TGA thermal analyzer in the Central Laboratory at METU, Ankara.

Preparation of the ligands

Synthesis of 1,1′-[butane-1,4-diylbis(oxybenzene-4,1-diyl)]diethanone (II)

This compound was synthesized as described previously.[6] A mixture of 4-hydroxyacetophenone (*I*) (10 mmol, 1.36 g), 1,4-dibromopropane (5 mmol, 1.08 g), and dry K_2CO_3 (10 mmol, 1.38 g) in 40 mL of acetone was refluxed with stirring for 15 h and poured into 200 mL of cold water. The white precipitate formed was filtered and washed with water and finally recrystallized from acetone-water. Yield 74.1%; mp 146 °C; IR (KBr) (ν, cm^{-1}) 1677 (C=O), 1247 and 1176 (C-O-C); ^{1}H-NMR (DMSO-D6) δ 2.49 (s, 6H, CH_3), 2.03 (qn, 4H, J=6.04, CH_2), 4.10 (t, 4H, J=6.00, OCH_2), 6.87 and 7.53 (dd, 8H, J=7.02, Ar-H); ^{13}C-NMR (DMSO-D6, ppm) 197.3 (C1), 163.6 (C2), 130.6 (C3), 131.4 (C4), 114.2 (C5), 68.1 (C6), 27.7 (C7), 26.1 (C8); Analysis (% Calculated/found) for $C_{20}H_{22}O_4$, C: 73.53/73.24, H: 6.13/6.29.

Synthesis of (1Z,1′Z)-1,1′-{butane-1,4-diylbis[oxybenzene-4,1-diyl(1Z)eth-1-yl-1-ylidene]}dihydrazine (III)

This compound was synthesized as described previously.[6] Hydrazine hydrate (5 mL) was added to *II* (10 mmol, 3.12 g) in 5 mL of absolute ethanol. The mixture was refluxed while stirring for 4 h. The compounds that precipitated during refluxing were filtered and washed with distilled water. The pure bis(hydrazine) was obtained by recrystallization from hot ethanol. Yield 75%; mp 200 °C; IR (KBr) (ν, cm^{-1}) 3323 and 3185 (NH_2), 1604 (C=N), 1252 and 1173 (C-O-C); ^{1}H-NMR (DMSO-D6) δ 2.03 (s, 6H, CH_3), 1.97 (qn, 4H, J=5.98, CH_2), 4.10 (t, 4H, J=6.10, OCH_2), 6.12 (s, 4H, NH_2), 6.84 and 7.88 (dd, 8H, J=7.00, Ar-H); ^{13}C-NMR (DMSO-D6, ppm) 159.5 (C1), 133.0 (C2), 128.2 (C3), 110.4 (C4), 115.3 (C5), 67.2 (C6), 26.9 (C7), 12.4 (C8); Analysis (% Calculated/found) for $C_{20}H_{26}N_4O_2$, C: 67.52/67.36, H: 7.31/7.09, N: 15.75/15.93.

Synthesis of 1,4-bis{4-{(1E)-1-((2E)-(2-hydroxybenzene–2-ylmethylidene) hydrazonylidene)ethyl} phenoxy}butane (H_2L^1)

Salicylaldehyde (2 mmol, 0.244 g) dissolved in ethanol (10 mL) was added dropwise to a solution of dihydrazine (*III*) (1 mmol, 0.354 g) with 2 drops of glacial acetic acid in hot ethanol (30 mL). The reaction mixture was

stirred while refluxing for 1 h. The compounds that precipitated during refluxing were filtered off and washed several times with water and hot ethanol, and dried in air at room temperature. Crystallization of the crude products from ethanol–chloroform furnished bis-azine compound. Yield 75%; mp 189 °C; UV (CH_2Cl_2, nm) 319.0; IR (KBr) (ν, cm^{-1}) 3250-2600 (O-H), 1608 (C=N-N=C), 1256 and 1176 (C-O-C); ^{1}H-NMR (DMSO-D6) δ 2.52 (s, 6H, CH_3), 2.22 (qn, 4H, J=6.00, CH_2), 4.32 (t, 4H, J=6.01, OCH_2), 6.98 and 7.98 (dd, 8H, J=6.95, Ar-H), 7.45-8.01 (m, 8H, Ar-H), 8.78 (s, 2H, HC=N), 12.05 (s, 2H, OH); ^{13}C-NMR (DMSO-D6, ppm) δ 165.2 (C1), 160.1 (C2), 132.4 (C3), 131.9 (C10), 130.2 (C15), 128.9 (C14), 121.3 (C5), 119.5 (C4), 117.0 (C12), 115.1 (C14), 65.6 (C6), 285 (C7), 15.8 (C8), 1622 (C9), 161.1 (C11); Analysis (% Calculated/found) for $C_{34}H_{34}O_4N_4$, C: 72.51/72.77, H: 6.04/5.83, N: 9.93/10.32.

Synthesis of 1,4-bis{4-{(1E)-1-((2E)-(pyridine–2-ylmethylidene)hydrazonylidene)-ethyl}phenoxy} butane (L^2)

Pyridine-2-carbaldehyde (2 mmol, 0.214 g) dissolved in ethanol (10 mL) was added dropwise to a suspension of dihydrazine (*III*) (1 mmol, 0.354 g) with 2 drops of glacial acetic acid in ethanol (40 mL) in room temperature. The reaction mixture was stirred for a further 4 h and was kept overnight. The yellow precipitated bis-azine was collected by filtration, and washed with water and ethanol. The pure ligand was collected by crystallization from ethanol–chloroform. Yield 74%; mp 170 °C; UV (CH_2Cl_2, nm) 347.0 and 317.0; IR (KBr) (ν, cm^{-1}) 1607 (C=N-N=C), 1554 (C=N), 1245 and 1180 (C-O-C); ^{1}H-NMR (DMSO-D6) δ 2.55 (s, 6H, CH_3), 2.09 (qn, 4H, J=6.00, CH_2), 4.20 (t, 4H, J=6.04, OCH_2), 6.97 and 7.91 (dd, 8H, J=7.02 Ar-H), 7.30-8.68 (m, 8H, Ar-H), 8.46 (s, 2H, HC=N); ^{13}C-NMR (DMSO-D6 ppm) 163.98 (C1), 150.39 (C2), 131.77 (C3), 137.50 (C4), 115.21 (C5), 67.18 (C6), 26.89 (C7), 13.76 (C8), 161.44 (C9), 155.17 (C10), 158.60 (C11), 125.49 (C12), 129.17 (C13), 122.30 (C14). Analysis (% Calculated/found) for $C_{32}H_{32}N_6O_2$ C: 72.10/72.39, H: 6.01/6.23, N: 15.77/16.19.

Synthesis of binuclear complexes of H_2L^1 {$[M(L^1)]_2.2H_2O$, (M= Ni(II) or Cu(II))}

A solution of $Ni(AcO)_2{\cdot}4H_2O$ (0.249 g, 1 mmol) or $Cu(AcO)_2{\cdot}H_2O$ (0.20 g, 1 mmol) in EtOH (10 mL) (for Ni(II) complex) or in MeOH (10 mL) for (Cu(II) complex) was added dropwise over 1 h to a hot solution containing bis(azine) H_2L^1 (0.563 g, 1 mmol) and NaOH (0.80 g, 2 mmol) in EtOH (30 mL) (for Ni(II) complex) or triethylamine (2 mmol, 0.202 g) in acetone (30 mL) (for Cu(II) complex) under stirring. The reaction mixture was refluxed for 5 h. The precipitated complexes were filtered off, and washed with water and chloroform.

For $[Ni(L^1)]_2.2H_2O$: Light green complex; yield: 76%; mp: 310 °C; μ_{eff} = 3.28 B.M.; FT-IR (KBr, cm^{-1}): 3435 b (O–H), 1602 s (C=N-N=C), 1255 s and 1176 m (C-O-C). Analysis (% Calculated/found) for $C_{68}H_{68}N_8Ni_2O_{10}$ C: 64.02/64.32, H: 5.33/5.42, N: 8.79/8.51, Ni: 9.21/9.48.

For $[Cu(L^1)]_2.2H_2O$: Brown complex; yield: 73%; mp: 251 °C; μ_{eff} = 1.45 B.M.; FT-IR (KBr, cm^{-1}): FT-IR (KBr, cm^{-1}): 3416 b (O–H), 1601 s (C=N-N=C), 1246 s and 1174 m (C-O-C). Analysis (% Calculated/found) for $C_{68}H_{68}Cu_2N_8O_{10}$ C: 65.37/64.94, H: 5.29/5.11, N: 8.72/8.49, Cu: 9.89/10.12.

Synthesis of mononuclear complexes of L^2 {$[M(L^2)Cl_2]$ (M= Ni(II) or Cu(II))}

A solution of 1 mmol metal(II) chloride [$CuCl_2 \cdot 2H_2O$ (0.17 g) or $NiCl_2 \cdot 6H_2O$ (0238 g)] in MeOH (10 mL) was added to a hot solution containing bis-azine ($L^2 = 0.519$ g, 1 mmol) and MeOH (25 mL) with stirring. The reaction mixture was refluxed for 5 h, and then the volume was reduced to ∼10 mL under reduced pressure. On standing overnight, the complexes separated, and were collected by filtration and washed with benzene and chloroform and finally water.

For $[Ni(L^2)Cl_2].H_2O$: Green complex; yield: 88%; mp: 245 °C; μ_{eff} = 2.93 B.M.; UV (EtOH, nm); 310.5, 202.5; FT-IR (KBr, cm^{-1}): 3350 b (O–H), 1596 s (C=N-N=C), 1509 m ($C{=}N_{pyridine}$), 1251 s and 1176 m (C-O-C). Analysis (% Calculated/found) for $C_{32}H_{34}Cl_2N_6NiO_3$ C: 56.45/56.69, H: 4.99/4.82, N: 12.35/12.13, Ni: 8.63/8.38.

For $[Cu(L^2)Cl_2].2H_2O$: Brown complex; yield: 87%; mp: 193 °C; μ_{eff} = 1.68 B.M.; UV (EtOH, nm) 371, 266.50; FT-IR (KBr, cm^{-1}): 3435 b (O–H), 1599 s (C=N-N=C), 1507 m ($C{=}N_{pyridine}$), 1263 s and 1174 m (C-O-C). Analysis (% Calculated/found) for $C_{32}H_{36}Cl_2CuN_6O_4$ C: 54.62/54.28, H: 5.12/5.19, N: 11.95/12.10, Cu: 9.03/8.85.

DNA binding

Electronic absorption titrations

All the experiments involving the interaction of the complexes with CT-DNA were carried out in water buffer containing 5 mM tris[(hydroxymethyl)-aminomethane] and 50 mM NaCl, and adjusted to pH 7.3 with HCl. The solution of CT-DNA in the buffer gave a ratio of UV absorbance of 1.8-1.9:1 at 260 and 280 nm, indicating that the CT-DNA was sufficiently free of protein.[22] The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm.[23] Appropriate amounts of metal complexes were dissolved in a solvent mixture of 1% DMF and 99% tris–HCl buffer. Absorption titration experiments were performed by maintaining the metal complex concentration as constant while gradually increasing the concentration of the CT-DNA within 0-75 μM.

DNA cleavage

pBR322 plasmid DNA was used for all cleavage activities. In a typical experiment, 7 μL of plasmid DNA (50 ng/μL) was mixed with the 60 μM solution of complexes solved in DMF. Then 5 μL of H_2O_2 (5 mM) was added to the mixture for oxidation of reactant. Finally the reaction mixture was diluted with the Tris buffer (100 mM Tris, pH 8) to a total volume of 30 μL. After that the reaction mixtures were incubated at 37 °C for 1 h. Samples (20 μL) were then incubated at 37 °C and loaded with 4 μL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 10 mmol EDTA), on 1% agarose gel containing 1 μg/mL of EtBr. The gel was run at 70 V for 45 min in TAE buffer and photographed in UV light.

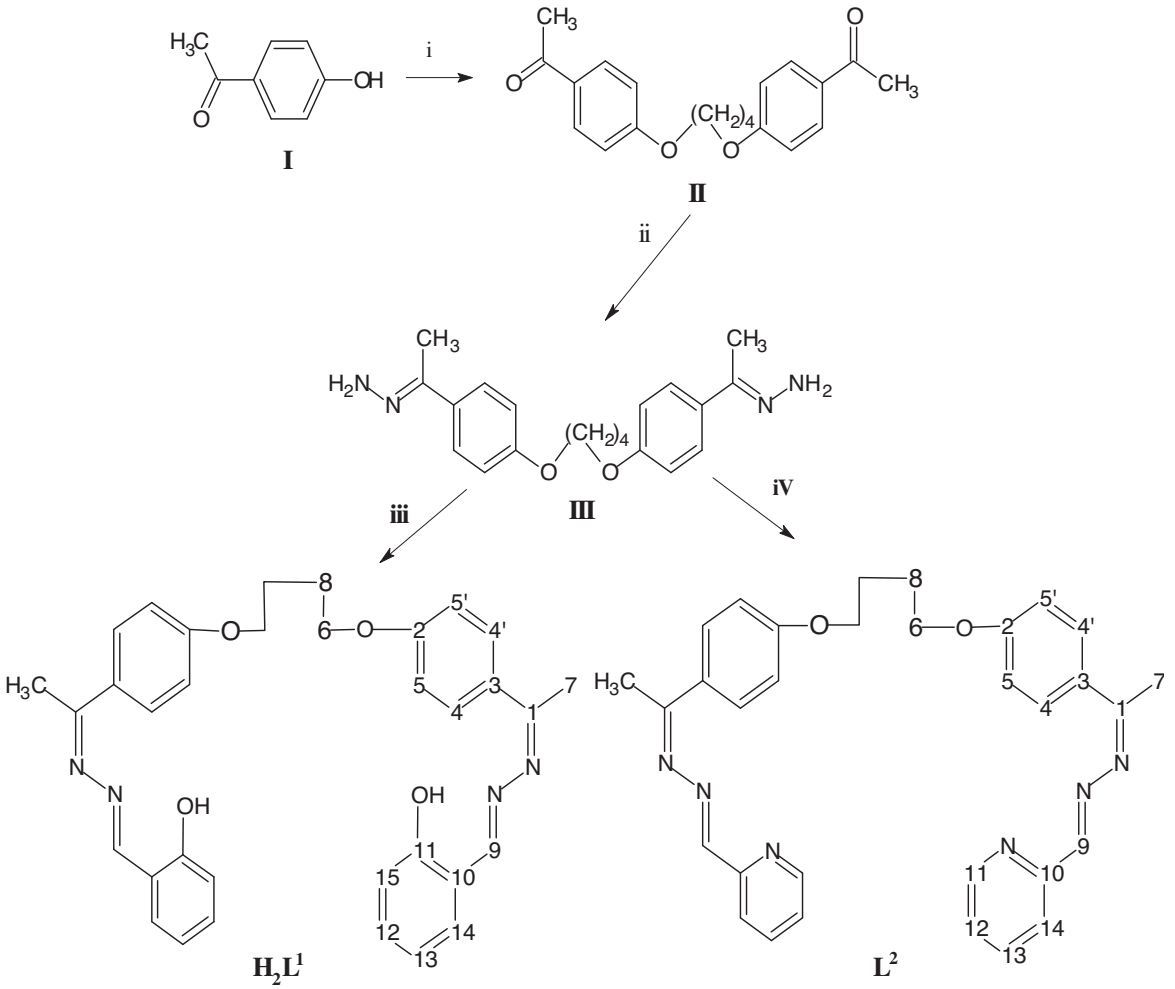
Solvent metal extraction

Picrate and/or dichromate extraction experiments were performed following Pedersen's procedure.[24] For this, 10 mL of 2.5×10^{-5} M aqueous picrate solution (Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) or 1

$\times\ 10^{-4}$ M aqueous dichromate solution (pH of dichromate solution has been maintained by 0.01 M KOH/HCl solution) and 10 mL of $1\ \times\ 10^{-3}$ M solution of bis-azine in CH_2Cl_2 were vigorously agitated in a stoppered glass tube with a mechanical shaker for 2 min and then magnetically stirred in a thermostated water-bath at 25 °C for 1 h, and finally left standing for an additional 30 min. The concentration of picrate/dichromate ion remaining in the aqueous phase was then determined spectrophotometrically as previously described.[25] Blank experiments showed that no picrate/dichromate extraction occurred in the absence of bis(azine). The percent extraction ($E\%$) was calculated as follows:

$$E\% = [(A_0 - A)/A_0] \times 100 \tag{1}$$

where A_0 and A are the concentrations of the metal picrate/dichromate before and after the extraction, respectively.



Scheme. i, Br-$(CH_2)_4$-Br and K_2CO_3, acetone, reflux 15 h; **ii**, $NH_2NH_2 \cdot H_2O$, EtOH, reflux 4 h.; **iii,** salicylaldehyde, EtOH, reflux 4 h.; **iv,** pyridine-2-carbaldehyde, EtOH, room temperature.

Results and discussion

Synthesis

The 1,1′-[butane-1,4-diylbis(oxybenzene-4,1-diyl)]diethanone (*II*) was synthesized by refluxing 4-hydroxyacetophenone with 1,4-dibromopropane stoichiometrically in the presence of dry K_2CO_3 in acetone. The reaction of this compound with hydrazine monohydrate gave bis-dihydrazine, (1*E*,1′*E*)-1,1′-{butane-1,4-diylbis[oxybenzene-4,1-diyl(1*E*)eth-1-yl-1-ylidene]}dihydrazine (*III*). The bis-azine ligands were prepared by the condensation of bis-(diyhdrazine) with salicylaldehyde and pyridine-2-carbaldehyde in the molar ratio 1:2. The reactions proceeded smoothly, producing the corresponding bis(azine)s in good yield (Scheme). The ligands are soluble in common organic solvent but insoluble in water. The structures of the ligand were elucidated by elemental analyses, FTIR, electronic absorption, and ^{1}H- and ^{13}C-NMR. The complexes were synthesized by reacting with the ligands with metal(II) salt in the molar ratio of 1:1. Attempts to isolate crystals suitable for single X-ray diffraction were unsuccessful. Therefore, the overall geometry of the complexes was inferred from the thermal, elemental analysis, magnetic susceptibility IR, and electronic spectra.

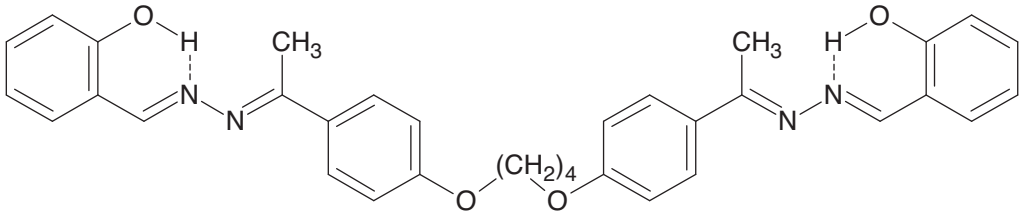


Figure 1. Intramolecular hydrogen bonding of H_2L^1.

^{1}H- and ^{13}C-NMR spectroscopy

In order to identify the structures of the bis-azine ligands, the ^{1}H- and ^{13}C-NMR spectra were recorded and the chemical shifts are given in the experimental section. As expected for the *para*-disubstituted benzene, the aromatic protons of 1,4-bis(4-acetylphenoxy)butane (*II*) give doublet-doublet peaks. The ^{1}H-NMR spectra of this compound exhibit a singlet at 2.49 ppm for CH_3 protons, a triplet at 4.10 ppm for OCH_2-C protons, and a quintet at 2.03 for C-CH_2-C protons. The ^{1}H-NMR spectrum of the dihydrazine (*III*) shows an extra D_2O exchangeable peak at 6.12 ppm as a singlet which is attributed to the (NH_2) group's protons in the molecule Furthermore, in the spectrum of bis(azine)s this peak disappears indicating that this group reacts to the carbonyl group of the aldehyede to give an asymmetric bis-azine. In the ^{1}H-NMR spectra of the new bis-azine ligands, the chemical shifts observed at 8.78 and 8.46 ppm as a singlet are assigned to the protons of the imine groups (CH=N) for H_2L^1 and L^2 ligands, respectively The OH resonance in the ^{1}H-NMR spectra of H_2L^1 appears as a singlet at 12.05 ppm. The shift in phenolic OH proton absorption of this azine to lower frequency may be explained by strong intermolecular hydrogen bonding (Figure 1). The other obtained values for ^{1}H-NMR chemical shifts of these compounds are given in the experimental section. These data are in agreement with those previously reported for similar compounds.[7−11,26−31]

The ^{1}H-NMR spectral data of the new bis-azine are also supported by the ^{13}C-NMR spectrum. In the ^{13}C-NMR spectrum of compound *II*, the chemical shift observed at 197.3 ppm is attributed to the C(1)

atom of the carbonyl group. On the other hand, in the ^{13}C-NMR spectrum of the dihydrazine *III* this signal disappears and a new chemical shift is observed at 159.5 ppm for the carbon atom C(1) of the azomethine group indicating that hydrazine and the carbonyl group were condensed to give dihydrazine (*III*). The other peaks observed for compounds *II* and *III* are given in the experimental section. Characteristic chemical shifts in the azine C(1)=N-N=(9)C carbon atoms confirming the structures of the synthesized azine ligands were obtained at 165.2-162.2 and 1639-161.4 ppm for H_2L^1 and L^2, respectively. In the ^{13}C-NMR spectrum of the ligands, the signals at 161.10 ppm and 155.17 ppm are attributed to the carbon atom of the C(11)-OH bond of salicylaldehyde moiety of H_2L^1 and the carbon atom of C(10)=N bond of the pyridine ring of L^2. The other chemical shifts observed for aromatic and aliphatic carbon atoms are given in the experimental section. These data are in good agreement with those previously reported for similar compounds.[8–11,18,32,33] These results strongly suggest that the proposed bis-azine ligands have been formed. Due to the paramagnetic nature of the complexes, their ^{1}H- and ^{13}C-NMR spectra could not be obtained.

IR spectroscopy

The IR spectrum of 1,4-bis(4-acetylphenoxy)butane (*II*) shows C=O stretching vibration at 1677 cm^{-1}. However, in the IR spectra of dihydrazine (*III*) this band disappears and a new vibration band for azomethine >C=N is observed at 1604 cm^{-1}, indicating that complete condensation takes place. Furthermore, the peaks observed at 3323 and 3185 cm^{-1} are attributed to symmetric and asymmetric stretching vibrations of the NH_2 group for *III*. The IR spectrum of the bis-azine ligands did not display bands for the NH_2 group after the condensation reactions of dihydrazine *III* with salicylaldehyde and pyridine-2-carbaldehyde. Furthermore, new medium bands at 1608 and 1607 cm^{-1} are observed for characteristic stretching vibration of the azine group (>C=N-N=C<). These results indicate that the formation of asymmetric bis-azine was completed. Furthermore, a broad stretching vibration observed in the 2600-3250 cm^{-1} region is assigned to the intermolecular H-bonding vibration (O–H$\cdots$O) in the IR spectra of H_2L^1, whereas in the IR spectrum of L^2 the peak observed at 1541 cm^{-1} is attributed to stretching vibration of the (C=N) pyridine group.[2–4,27] The other characteristic IR peaks of bis-azine compounds synthesized in this work are given in the experimental section. These values are in accord with those previously reported for such compounds.[8–11,32,34]

It is well known that the coordination of the ligand to metal ions through imine nitrogen atoms reduces the electron density of this bond, thus lowering the C=N symmetric stretching frequency. Therefore, these bands of free ligands were moved to lower frequency after complexation indicating that bis(azine) ligands are coordinated through their azine groups. In the case of H_2L^1 ligand, the hydrogen bonded phenolic OH stretching vibration of free ligand disappears upon the formation of the complexes, indicating the deprotonation of the phenolic proton and participation complexation.[7,28,35] According to the IR spectra of free ligands and their complexes, it is concluded that H_2L^1 ligand acts as a dianionic tetradentate ligand coordinating to azine and salicylaldehyde moiety whereas L^2 ligand acts as a neutral tetradentate ligand coordinating to both azine and pyridine imine groups. A broad new band arises between 3350 and 3450 cm^{-1}, which can be assigned to the stretching vibration of the water molecules in the spectra of all metal complexes indicating that they have water molecules.[6,7,18]

Table 1. Thermal decomposition of metal complexes.

Complex	TG range (°C)	Mass loss estimated (%, calculated)	Assignment
$[Ni(L)]_2$	25-160	2.90 (2.82)	$2H_2O$
	160-305	7.60	Loss of functional group of azine-complex
	305-585	40.0	Loss of functional group of azine-complex
	585-950		Decomposition of remaining azine-complex and remaining ash
$[Cu(L)]_2$	25-115	2.90 (2.80)	$2H_2O$
	115-595	48.20	Loss of functional group of azine-complex
	595-950		Decomposition of remaining azine and remaining ash
$[Ni(L)Cl_2]$	25-110	2.50 (2.65)	H_2O
	110-310	10.70 (10.39)	2Cl
	310-500	24.60	Loss of functional group of azine-complex
	500-900		Decomposition of remaining ligand and ash
$[Cu(L)Cl_2]$	25-120	4.90 (5.12)	$2H_2O$
	120-320	9.80 (10.09)	2Cl
	300-450	6.60	Loss of functional group of azine-complex
	450-900		Decomposition of remaining ligand and ash

Thermal analyses

Thermogravimetry (TG) is a powerful method to determine complex stoichiometries. The thermal data of the complexes are given in Table 1. The thermogravimetric analysis (TGA) curves of all the complexes exhibit stepwise mass losses. TGA curves of double-stranded binuclear complexes are different from those of mononuclear complexes.

According to TGA curves, thermal degradation of mononuclear complexes $[M(L)Cl_2].nH_2O$ occurred in 4 to 5 steps. In the first step of decomposition, mononuclear complexes showed water molecule loss for nickel complex of 2.50% and copper complex of 4.90% below 120 °C. This low temperature loss confirms that the water molecules do not participate in coordination and are held in lattice voids. The weight loss of the second step was 10.70% and 9.8% for $[Ni(L)Cl_2]$ and $[Cu(L)Cl_2]$, respectively, within the temperature range 110-320 °C corresponding to the removal of the chloride anion attached to the metal ions.[36,37] The other steps of decomposition of mononuclear complexes correspond to the removal of the organic part of the ligand, leaving metallic ash as a residue.

The double-stranded binuclear nickel(II) complex shows the first step decomposition within the temperature range 25-160 °C. This high temperature loss confirms that the water molecules participate in coordination. On the other hand, binuclear copper(II) complex shows that the first step decomposition within the temperature range 25-115 °C corresponds to elimination of hydration water molecules. The decompositions of other steps are attributed to the loss of functional groups of the double-stranded binuclear azine complexes. The last step is due to breaking of the binuclear complexes, leaving metallic ash as a residue.

Magnetic moment studies

Magnetic susceptibility measurements of the complexes were carried at room temperature and provide information regarding their structures. These measurements indicate that all complexes are paramagnetic at ambient temperature. The observed magnetic moment values for mononuclear Cu(II) and Ni(II) complexes are 1.68 and 2.93 BM, respectively, which are within the range for mononuclear copper(II) and nickel(II) complexes. Room temperature effective magnetic moment values for the binuclear Cu(II) and Ni(II) complexes are 1.45 and 3.28 BM, respectively. It can be observed that in the case of binuclear copper(II) complexes these magnetic moment values of complexes are slightly lower than the theoretical value of 1.73 μ_B for one d^9 copper ion while the observed magnetic moment values for binuclear nickel(II) complexes are slightly higher than the theoretical value of 2.82 μ_B for one d^8 nickel ion. However, these magnetic moment values are lower than those expected for binuclear copper(II) and nickel(II) complexes. These abnormal values may be explained by weak antiferromagnetic intramolecular interactions between metal centers, resulting in a decrease in the magnetic moments.[18,38,39] Magnetic data show that both mono- and binuclear nickel(II) complexes, which are in an octahedral environment created by the additional axial coordination of the chloride and water molecules for mono- and binuclear complexes, respectively, adopt a high-spin configuration in the both mononuclear and double-stranded binuclear complexes (Figures 2 and 3).

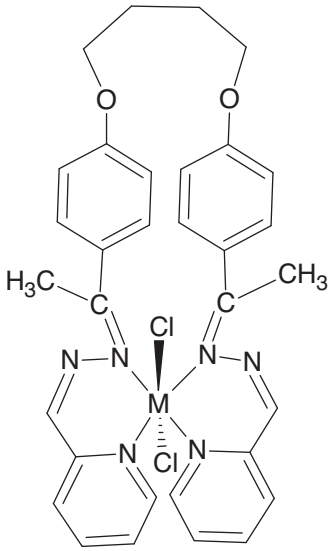


Figure 2. Proposed structure of the mononuclear complexes of L^2 [M=Ni(II) or Cu(II)].

Electronic absorption spectra

The electronic absorption spectral characteristics of the bis(azine) ligands in CH_2Cl_2 are given in the experimental section. The broad bands observed in the region of 319 and 317-347 for H_2L^1 and L^2, respectively, are attributed to $n \rightarrow \pi^*$ electronic transitions of the nonbonding electron pairs.[28,29,38]

Electronic absorption spectra of the mononuclear complexes of L^2 ligand were recorded in EtOH solutions. In the comparison of the electronic absorption of L^2 and its Ni(II) complexes, it is observed that the band appearing at 347 nm for L^2 shows a considerable hypsochromic shift (310.5 nm) while in the case of

Cu(II) complexes this band shows a considerable red shift (371.0), indicating that the ligand participates in complexation. These bands may be attributed to the charge transfer transitions. The other bands observed in the electronic spectra of the complexes are attributed to $\pi \rightarrow \pi^*$ electron transition. Unfortunately the expected weak d-d transition in the visible region for all complexes cannot be detected even with concentrated solution. It may be lost in the low energy tail of the charge transfer transition.[28,29,38,39]

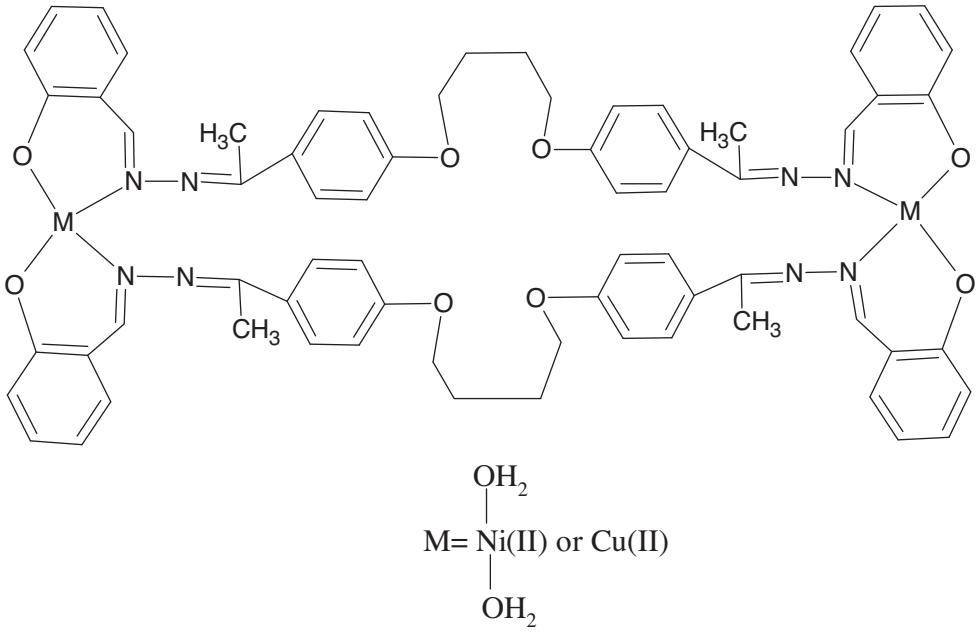


Figure 3. Proposed structure of the double-stranded binuclear complexes of H_2L^1 [M=Ni(II) or Cu(II)].

DNA binding studies

Electronic absorption titrations

Absorption titration is an effective method to examine the binding mode of DNA with metal complexes.[22,23,40,41] Therefore, the binding interaction of the mononuclear complexes with CT-DNA was monitored by UV-Vis spectroscopy. Unfortunately, the insolubility of the double-stranded binuclear complexes hinders the study of the DNA interaction activities of these complexes. Furthermore, no important DNA interactions were observed for any of the metal free bis(azine) ligands. In the case of intercalative binding mode, the π^* orbital of the complex can couple with the π orbital of the DNA base pairs, decreasing the $\pi \rightarrow \pi^*$ transition energy. This results in bathochromism. Moreover, the coupling π orbital is partially filled, thus decreasing the transition possibilities and resulting in hypochromism. In general, hypochromism and bathochromism are associated with an intercalative binding mode, because of strong stacking interactions between the aromatic chromophore of the complex and the base pairs of DNA. The extent of hypochromism and red shift is connected with the strength of the intercalative interactions.[42,43]

The absorption spectra of the mononuclear complexes recorded in the absence and presence of increasing amounts of CT-DNA are given in Figure 4. The absorption spectra of the complexes are affected by the increasing concentration of CT-DNA. The spectra show clearly that the addition of DNA causes hyperchromism with a red

shift. With increasing concentration of CT-DNA, the absorption bands of mononuclear copper(II) and nickel(II) complexes at 271.8 and 319.0 nm, respectively, exhibited hyperchromism (–33.99% and –3.35%), accompanied by a small red shift. Furthermore, the isobestic points were observed at 298.7 nm and 294.2 nm for $[Cu(L)Cl_2]$ and $[Ni(L)Cl_2]$, respectively. According to these data it may be concluded that both mononuclear complexes bind to DNA in classical intercalative mode. The binding constant, K_b, was determined by using the following equation:[41]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_o - \varepsilon_f) + 1/Kb(\varepsilon_o - \varepsilon_f) \quad (2)$$

where [DNA] is the concentration of DNA in base pairs; ε_a, ε_f, and ε_o correspond to $A_{obsd}/[M]$, the extinction coefficient of the complexes, and the extinction coefficient of the complex in the fully bound form, respectively; and K_b is the intrinsic binding constant. The ratio of the slope to intercept in the plot of [DNA] / $(\varepsilon_a - \varepsilon_f)$ versus [DNA] gives the value of K_b for complex. The binding constants of mononuclear copper complex are much higher than those of mononuclear nickel complexes (Table 2).

Table 2. Effect of CT-DNA on the absorbance bands and binding constant of the complexes and adducts.

Complex	λ_{max} (nm)		$\Delta\lambda$ (nm)	H (%)	K_b (M^{-1})
	Free	Bound			
$[Ni(L)Cl_2]$	319.0	319.4	0.4	–33.99	0.6×10^4
$[Cu(L)Cl_2]$	271.8	274.0	2.2	–3.35	0.1×10^5

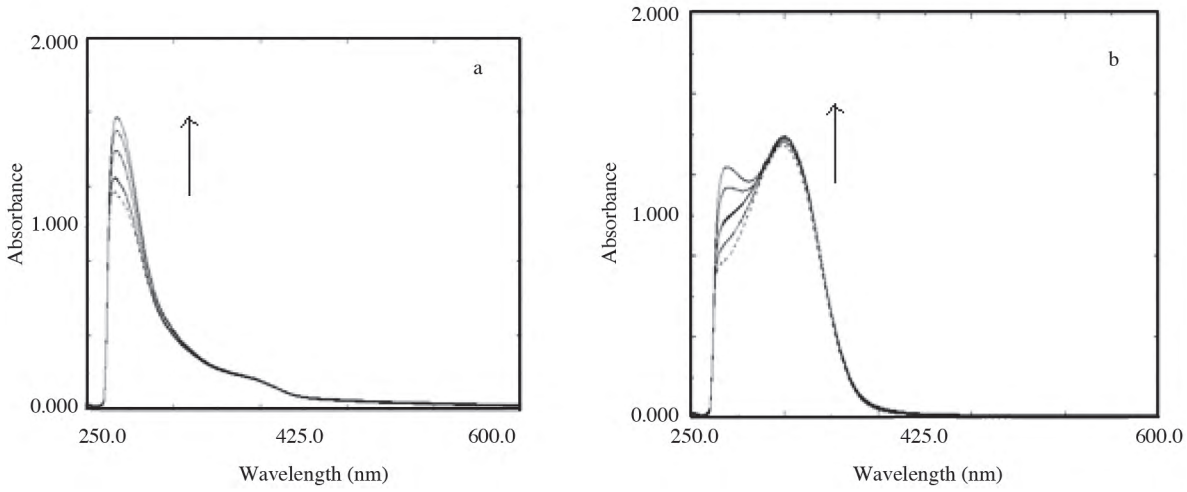


Figure 4. Electronic spectra of mononuclear Cu(II) and Ni(II) complexes in the presence of increasing amounts of CT-DNA. [CT-DNA] = 0-75 μM. (a): $[Cu(L)Cl_2]$, (b): $[Ni(L)Cl_2]$. (—) presence of CT-DNA, (- - -) absence of CT-DNA.

DNA cleavage studies

The effect of the synthesized mononuclear complexes in the presence of H_2O_2 on supercoiled pBR322 plasmid DNA was studied at 37 °C in TAE buffer. The complexes did not exhibit any DNA nuclease activity in the

absence of oxidant. DNA cleavage was controlled by relaxation of supercoiled circular form of pBR322 into the nicked circular and linear form. When circular pBR 322 DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand (nicking), the supercoiled form will relax to generate a slower-moving nicked form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated. The gel electrophoretic separations of plasmid pBR322 DNA induced by the complexes in the presence of H_2O_2 are given in Figure 3. Lane 2 in the figure shows the control DNA without any additives. Both mononuclear copper and nickel complexes showed significant nuclease activity on supercoiled circular DNA in the presence of H_2O_2, which, may be due to the reaction of hydroxyl radical with DNA. As seen in Figure 5, both complexes displayed different cleavage activities under the same physiological conditions. For complex $[Cu(L)Cl_2]$ (Figure 5, lane 1) the cleavage is found to be much more efficient: it cleaved the supercoiled circular form (Form I) to nicked circular form (Form II) and linear form (Form III) and the band of Form I also disappeared completely. It is clearly seen from Figure 5 that the percentage of the nicked DNA molecules is much higher than that of linear DNA molecules. On the other hand, mononuclear Ni(II) complexes showed much lower nuclease activities. It is converted slightly from Form I to Form II, but the supercoiled form is still seen (Figure 5, lane 3). The cleavage percentage of nicked form is very low. The different DNA cleavage efficiencies of the 2 mononuclear complexes may be due to the different binding affinity of the complexes to DNA. In addition, the type of metal ion may also affect the nuclease activity of the complexes[6,18,44–47]

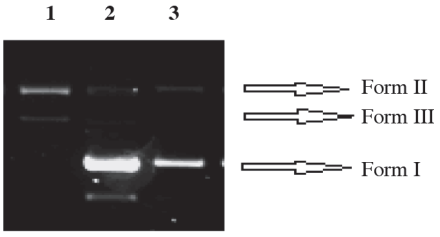


Figure 5. Oxidative cleavage of pBR322 DNA by Cu(II) and Ni(II) complexes. Lane 1; $[Cu(L)Cl_2]$ (60 μM), lane 2; DNA control, lane 3; $[Ni(L)Cl_2]$ (60 μM).

Solvent extraction studies

Metal cations

Schiff bases have found numerous applications as highly selective reagents for the separation and determination of a number of metal ions. The metal binding properties of bis-azine ligands were examined by solvent extraction of selected transition metal picrates [Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), Pb(II) and Ag(I)] from the aqueous to an organic phase under neutral conditions. After complete phase separation, the equilibrium concentration of picrate in the aqueous phase was determined spectrophotometrically. The percentages of the cations extracted (E%) by the bis-azine ligands are given in Table 3.

From the data in Table 3, it can be seen that H_2L^1 does not extract the transition metals except Hg(II) ions also transferring poorly from aqueous phase to organic phase. The strong intermolecular hydrogen bonding of this azine may obstruct extraction ability toward selected transition metals. On the other hand, L^2 ligand

shows a high extraction for Ag(I) and Hg(II) ions compared to the other metal ions. The percentage of Ag(I) cation extracted by L^2 is much higher than that of Hg(II) ions. This phenomenon may be explained by the hard soft acid–base principle. Because of having azine (>C=N-N=C<) and azomethine (C=N) groups this ligands acts as a soft base and, therefore, shows high affinity toward soft acids such as Ag(I) and Hg(II) ions. These results are in accord with recent findings.[6,7,20,27,48]

Table 3. Extraction percentages of metal picrates with bis-azine ligands.[a]

Ligand	Extracted metal cations							
	Cu^{+2}	Ag^{+1}	Co^{+2}	Ni^{+2}	Pb^{+2}	Hg^{+2}	Zn^{+2}	Cd^{+2}
H_2L^1	1.84	0.42	1.19	0.71	3.84	28.95	3.79	11.07
L^2	18.13	81.21	0.49	8.59	0.10	56.05	2.56	0.21

[a] Aqueous phase: [metal nitrate] = 1×10^{-2} M; [picric acid] = 2.5×10^{-5} M; organic phase: dichloromethane, [ligand] = 1×10^{-3} M; at 25 °C, for 1 h.

Anions

The binding efficiencies of the bis(azine) ligands have also been determined by solvent extraction of $Na_2Cr_2O_7$ from aqueous solution into dichloromethane at different pH values. The results are summarized in Table 4. An aqueous solution of $Na_2Cr_2O_7$ shows no extraction into a phase in the absence of the extractant. The data in Table 4 show that both bis(azine) ligands effectively extract dichromate anions at low pH. When the pH of the aqueous phase decreases, the extraction efficiencies of the azines increase. The best result was obtained with a pH of 1.5 for both ligands. Moreover, at this pH level, dichromate anion extraction ability of L^2 is higher than that of H_2L^1 probably because of the fact that L^2 has more >C=N groups.

Table 4. Extraction percentages of dichromate anions by bis-azine ligands.[a]

Ligand	pH				
	1.5	2.5	3.5	4.5	5.5
H_2L^1	71.10	33.44	19.07	14.27	5.37
L^2	84.59	44.81	38.75	30.49	16.80

[a] Aqueous phase: [$Na_2Cr_2O_7$] = 1×10^{-4} M; organic phase: dichloromethane, [ligand] = 1×10^{-3} M at 25 °C, for 1 h.

It is known that dichromate anions exist in more acidic aqueous solutions as the $HCr_2O_7^-/Cr_2O_7^{2-}$ pair form. At higher acidic conditions $HCr_2O_7^-$ and $Cr_2O_7^{2-}$ dimers become the dominant Cr^{6+} form. It seems to us that the bis-azine ligand form complexes with both $HCr_2O_7^-$ and $Cr_2O_7^{2-}$ ions. The dichromate anions are principally in their protonated form $HCr_2O_7^-$ in aqueous solutions having a lower pH.

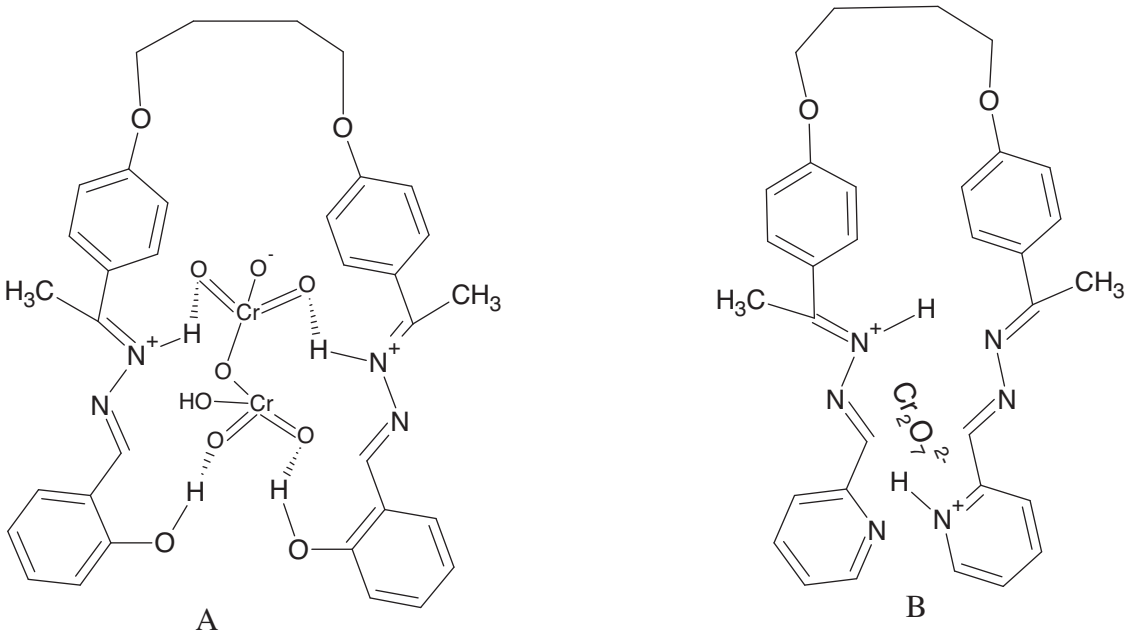


Figure 6. Proposed interactions of bis-azine ligands with dichromate anions (A for H_2L^1 and B for L_2).

L^2 ligand possesses azine and azomethine moieties, which lead to a hydrogen bond with dichromate anion. Therefore, dichromate anions can be transferred as a hydrogen-bonded ion pair interaction from the aqueous to the organic phase by the bis(azine) ligand. Following proton transfer to the nitrogen atoms of azine and pyridine groups in the ligand, an ion pair complex is formed between L^2 and $Cr_2O_7^{-2}/HCr_2O_7$ in the 2-phase extraction system.[6,7,20,49,50] Furthermore, a similar complex can be also formed in the 2-phase extraction system between the dichromate anion and oxygen atom of salicylaldehyde units of H_2L^1. As a consequence, based on the above results it has been concluded that azine, pyridine, and salicylaldehyde units probably play an important role in transferring the dichromate anions from aqueous phase to organic phase (Figure 6).

Conclusions

Two kinds of Cu(II) and Ni(II) complexes of new bis(azine)s derived from the condensation of (1*E*,1′*E*)-1,1′-{butane-1,4-diylbis[oxybenzene-4,1-diyl(1*E*)eth-1-yl-1-ylidene]}dihydrazine with salicylaldehyde and pyridine-2-carbaldehyde have been synthesized and characterized using different spectroscopic techniques. A ligand-to-metal ratio reaction of L^2 with metal(II) chloride gives the mononuclear complexes $[M(L^2)Cl_2]$, whereas a 1:1 molar ratio reaction of H_2L^1 with metal(II) acetate favors the formation of double-stranded binuclear complexes $[M_2(L^1)_2]$. On the basis of the elemental analyses, and stoichiometric and spectroscopic studies discussed above, it has been concluded that the H_2L^1 acts as a tetradentate dianionic N-O/N'-O' ligand that binds to metal ions through the nitrogen atom of azine and the oxygen atom of the salicylaldehyde hydroxyl group. On the other hand, the L^2 behaves as a tetradenteate neutral N-N'/N-N' ligand coordinating through the nitrogen atoms of azine and pyridine imine groups. The liquid-liquid extraction of selected transition metal cations [Cu(II), Ag(I), Co(II), Ni(II), Pb(II), Hg(II), Zn(II), Cd(II)] and dichromate anions from the aqueous

phase to the organic phase was carried out by using bis(azine) ligands. It was found the L^2 ligand has strong affinity toward Ag(I) cation and can be used for silver recovery. On the other hand, H_2L^1 ligand does not have affinity toward the selected transition metal ions. Both bis(azine) ligands are an effective extractant for dichromate anion at low pH. This can make these ligands, especially H_2L^1, suitable selective separating agents for dichromate anions. The DNA binding results showed that mononuclear complexes can bind to CT-DNA due to the chelating effect. The mononuclear complexes, especially Cu(II) complex, bind strongly to DNA, probably via an intercalation mechanism. We also conclude that all mononuclear complexes show nuclease activity in the presence of hydrogen peroxide as co-oxidant. Mononuclear copper(II) complex is more efficient in the cleavage of pBR322 DNA than the nickel(II) complex.

Acknowledgement

We thank the Scientific Research Projects Foundation of Muğla University for its financial support of this work.

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