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# Homo- and heteropolynuclear copper(II) complexes containing a new diimine–dioxime ligand and 1,10-phenanthroline: synthesis, characterization, solvent-extraction studies, catalase-like functions and DNA cleavage abilities

**Bülent Dede*, İsmail Özmen, Fatma Karipcin and Mustafa Cengiz**

**A series of homo-, heterodinuclear and homotrinuclear copper(II) complexes containing a new Schiff base ligand and 1,10-phenanthroline were synthesized. Based on results of elemental analyses, FTIR, $^{1}$H- and $^{13}$C-NMR spectra, conductivity measurements and magnetic susceptibility measurements, the complexes had general compositions {$[Cu(L)(H_2O)M(phen)_2](ClO_4)_2$ [M = Cu(II), Mn(II), Co(II)]} and {$[Cu_3(L)_2(H_2O)_2](ClO_4)_2$}. The metal : L : phen ratio is 2 : 1 : 2 for the dinuclear copper(II) complexes and the metal : L ratio was 3 : 2 for the trinuclear copper(II) complex. The liquid–liquid extraction of various transition metal cations [Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), Cd(II), Hg(II)] from the aqueous phase to the organic phase was carried out using the diimine–dioxime ligand. It was concluded that the ligand can effectively be used in solvent extraction of copper(II) from the aqueous phase to the organic phase. Furthermore, catalytic activitiy of the complexes for the disproportionation of hydrogen peroxide was also investigated in the presence of imidazole. Dinuclear copper(II)–manganese(II) complex has some similarity to manganese catalase in structure and activity. The interaction between these complexes and DNA has also been investigated by agarose gel electrophoresis; we found that the homo- and heterodinuclear copper complexes can cleave supercoiled pBR322 DNA to nicked and linear forms in the presence of $H_2O_2$.** 

**Keywords:** copper(II) complexes; polynuclear complexes; liquid–liquid extraction; catalytic disproportionation; DNA cleavage

## Introducion

Schiff base metal complexes have attracted great attention over the past decades as a result of their facile synthesis, wide application, the accessibility of diverse structural modifications, biological modeling applications, catalysis, design of molecular ferromagnets and materials chemistry.[1–6] These complexes are biologically important species that have numerous applications, such as in the treatment of cancer, as antibactericide agents, antivirus agents, fungicide agents and for other biological properties.[7–10]

Copper, cobalt and manganese are bioessential elements. More than a dozen enzymes that depend on copper for their activity have been identified; the metabolic conversions catalyzed by all of these enzymes are oxidative.[18] The cobalt complexes of tetradentate Schiff base ligands have been widely used to mimic cobalamine (B12) coenzymes.[10,19] Manganese ions play an important role in biological redox enzymes of many microorganisms, plants, and animals, and are exemplified as oxygen evolving centers of photosystem II, superoxide dismutases (SOD) and catalases (CAT).[10,20,21] Transition metal complexes with their efficient DNA binding and cleavage properties under physiological conditions is currently the subject of intense investigation in the fields of chemistry, biology and medicine.[11–15] This kind of study has become a very important field in the development of DNA molecule probes and chemotherapeutics in recent years.[10–18] In order to find anticarcinogens that can recognize and cleave DNA, researchers have synthesized and developed many kinds of complexes.

The copper and cobalt complexes of different ligands have attracted much attention due to their high nucleolytic efficiency, which is able to break the DNA chain in the presence of $H_2O_2$ and reducing agents.[18,22–28] However, at present, few articles about DNA-binding studies of homo- and heteropolynuclear copper(II) or cobalt(II) complexes have been reported.[29–33]

Transition metal ions such as Pb(II), Cd(II) and Hg(II) are recognized as highly toxic, which makes their presence in environmental waters or soils undesirable. These metals can accumulate in the environment and produce toxic effects in plants and animals even at very low concentrations. On the other hand some transition metals such as Co(II), Ni(II), Cu(II) and Zn(II) are bioessential and there are numerous metalloenzymes containing these elements. However, these

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elements also produce toxic effects in living things at high concentrations. Therefore, separation of these trace metals is vital due to the potential health and ecological hazzard.[34] The most widely used techniques for the separation and preconcentration of trace amounts are extraction,[35] precipitation[36] and chelating resins.[37] Furthermore in recent decades, many complex systems have been synthesized to mimic SOD-like activity.[38–40] SOD can protect cells from the toxic effects of superoxide ion from $O_2^-$ to $H_2O_2$ and $O_2$[41] by catalyzing the dismutation reaction of eqn (1). However, SOD-like model complexes have been investigated as catalysts to dismutate $H_2O_2$ as described in eqn (2).

$$2O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2 \quad (1)$$

$$2H_2O_2 \longrightarrow 2H_2O + O_2 \quad (2)$$

Since $H_2O_2$ is harmful to human cells and may cause a variety of pathological consequences,[42–44] a good SOD-model complex should first be a good catalase-like model complex.[45]

Herein we report the preparation, characterization and the extraction ability of the Schiff base ligand, which contains imine and oxime groups and homo- and heteropolynuclear copper(II) complexes of the ligand. Furthermore, their catalytic activity for the disproportionation of hydrogen peroxide and their DNA cleavage activities is also discussed. This result is helpful for understanding the binding mode of the complex to DNA further, and developing new useful DNA probes.

## Experimental

Chemicals and solvents were obtained from the commercial sources (Acros Organics, Aldrich, Fluka, Merck, Sigma) and used without further purification. DNA (supercoiled pBR 322) was purchased from Fermantas. 1-(Biphenyl)-2-hydroxyimino-2-(pyrrolidino)-1-etanone ($HL^1$) was synthesized according previously published procedures.[46–49] This compound was characterized by elemental analysis (C, H, N), melting point measurement, FTIR and NMR spectroscopies.

Measurements of $^1$H-NMR and $^{13}$C-NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 300 MHz spectrometer using TMS as an internal standart. A Shimadzu IRPrestige-21 FT-IR spectrophotometer was used to record infrared spectra of all compounds using KBr disks (4000–400 $cm^{-1}$). Elemental analyses (C, H, N) were performed using a Leco 932 CHNS analyzer and metal contents were estimated on a Perkin Elmer Optima 5300 DV ICP-OES spectrometer. Conductivity measurements of $10^{-3}$ M DMF solutions of prepared complexes were determined at room temperature using a Optic Ivymen System conductivity meter. The UV–vis measurements were performed on a Perkin Elmer λ20 UV–vis spectrometer. Determination of the melting points was performed using an electrothermal model IA 9100. The magnetic susceptibility measurements were done on a Sherwood Scientific Magnetic Susceptibility Balance (Model MX1) at room temperature.

### Synthesis of ligand [$H_2L$ (1)]

To a solution of absolute ethanol (10 ml) of $HL^1$ (30 mmol) was added diethylenetriamine (DETA) (1.547 g, 15 mmol) in absolute ethanol (10 ml). The content was stirred for 2 h at room temperature. The compound which precipitated was filtered off and washed several times with $Et_2O$ and dried over $P_2O_5$.

$^1$H NMR ($CDCl_3$, ppm): 8.45 (s, 2H, O–H), 7.33–7.79 (m, 18H, Ar–H), 6.57 (s, 1H, N–H), 3.65 (t, 4H, -$CH_2$-), 1.42 (t, 4H, -$CH_2$-), 3.47 (m, 8H, -$CH_{2\text{-pyr}}$), 1.89 (m, 8H, -$CH_{2\text{-pyr}}$). $^{13}$C NMR ($CDCl_3$, ppm): 173.76 (C6), 145.12 (C1), 125.67–142.17 (C7-C18), 61.83 (C19), 50.76 (C2 and C5), 46.17 (C20), 24.42 (C3 and C4).

### Synthesis of Complexes

*Caution:* all the perchlorate salts reported here are potentially explosive, therefore should be handled with care.

*Synthesis of [Cu($H_2L$)·($H_2O$)]($ClO_4$)$_2$*

The copper(II) complexes were prepared in a similar manner.[49] A solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (370 mg, 1 mmol) in $Me_2CO$ (25 ml) was added to the ligand solution (1 mmol) in 30 ml of EtOH, and this mixture was refluxed with stirring for 1 h. After stripping off the excess solvent under reduced pressure, a crude oily product was obtained. The mononuclear copper(II) complex was used without further purification.

*Synthesis of [Cu(L)·($H_2O$)Cu(phen)$_2$]($ClO_4$)$_2$ (**2**)*

The mononuclear copper complex (1 mmol) was added to $Et_3N$ (101 mg, 1 mmol) in MeOH (25 ml) and the mixture was stirred for 0.5 h. The solution of $Cu(ClO_4)_2.6H_2O$ (370 mg, 1 mmol) in MeOH (10 ml) and 1,10-phenanthroline monohydrate (397 mg, 2 mmol) in MeOH (10 ml) was successively added to the resulting mixture, which was refluxed for 3 h. The product was filtered off, washed with $H_2O$, MeOH and $Et_2O$ and dried over $P_4O_{10}$.

*Synthesis of [Cu(L)·($H_2O$)Mn(phen)$_2$]($ClO_4$)$_2$ (**3**)*

The mononuclear copper complex (1 mmol) was mixed with $Et_3N$ (101 mg, 1 mmol) in MeOH (20 ml) and stirred for 0.5 h. The solution of $Mn(OAc)_2.4H_2O$ (268 mg, 1 mmol) in MeOH (10 ml) and 1,10-phenanthroline monohydrate (397 mg, 2 mmol) in MeOH (10 ml) were successively added to the resulting solution. A stoichiometric amount of $NaClO_4$ (123 mg, 1 mmol) was then added to the resulting mixture which was refluxed for 3 h. The product was filtered off, washed with $H_2O$, MeOH and $Et_2O$ and dried over $P_4O_{10}$.

*Synthesis of [Cu(L)·($H_2O$)Co(phen)$_2$]($ClO_4$)$_2$ (**4**)*

The mononuclear copper complex (1 mmol) was mixed with $Et_3N$ (101 mg, 1 mmol) in MeOH (20 ml) and stirred for 0.5 h. The solutions of $Co(OAc)_2.4H_2O$ (249 mg, 1 mmol) in MeOH (10 ml) and 1,10-phenanthroline monohydrate (397 mg, 2 mmol) in MeOH (10 ml) were successively added to the resulting solution. A stoichiometric amount of $NaClO_4$ (123 mg, 1 mmol) was then added to the resulting mixture which was refluxed for 5 h. The product was filtered off, washed with $H_2O$, MeOH and $Et_2O$ and dried over $P_4O_{10}$.

*Synthesis of [$Cu_3$($L$)$_2$·($H_2O$)$_2$]($ClO_4$)$_2$ (**5**)*

A mixture of mononuclear copper complex (2 mmol), $Cu(ClO_4)_2.6H_2O$ (370 mg, 1 mmol) and $Et_3N$ (202 mg, 2 mmol) in $Me_2CO$ (25 ml) was refluxed for 2 h. The resulting solution was filtered while hot and concentrated slowly. As the solution cooled a powder product precipitated. It was isolated with vacuum filtration, washed with $Et_2O$ and dried over $P_4O_{10}$.

### Solvent-extraction

A chloroform solution (10 ml) of ligand ($1 \times 10^{-3}$ M) and an aqueous solution (10 ml) containing $2 \times 10^{-5}$ M picric acid and $1 \times 10^{-2}$ M metal nitrate were shaken at 25 °C for 1 h contact time. An aliquot of the aqueous solution was taken and the ultraviolet spectrum was recorded. For each diimine–dioxime ligand, the extraction experiments and the absorbance measurements were repeated three times. Blank experiments showed that no picrate extraction occurred in the absence of ligand. The extractability of the metal cations [Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), Cd(II), Hg(II)] is expressed by means of the following equation:

$$\text{Extractability \%} = [(A_0 - A)/A_0] \times 100$$

where $A_0$ and $A$ are the absorbances in the absence and presence of ligand, respectively.

### Studies on Catalase-like Function

Volumetric measurements of evolved dioxygen during the reactions of the heterodinuclear complexes 2–5 with $H_2O_2$ were carried out as follows: a 50 ml three-necked round-bottom flask containing a solution of the complexes (0.005 mmol solid sample) in DMF (10 ml) was placed in a water bath (25 °C). One of the necks was connected to a burette and the others were stoppered by a rubber septum. While the solution was stirring, $H_2O_2$ (1.33 mmol, 0.150 ml) was injected into it through the rubber septum using a microsyringe. Volumes of evolved dioxygen were measured at 1 min time intervals by volumometry. In cases where imidazole (50 mg) was added this was introduced into the reaction vessel before the addition of $H_2O_2$ (in the absence of the imidazole the complexes were either inactive or very weak catalysts for this reaction).

### Cleavage of pBR 322 DNA

For the agarose gel electrophoresis experiments, 0.5 µg/µl supercoiled pBR322 DNA (0.5 µl) was treated with 1 µl of 1 mM the tested ligand and its complexes in DMF and 2 µl of 0.1 M Tris–HCl (pH 8.0) buffer in the absence and presence of 2 µl of 5.0 mM hydrogen peroxide as a co-oxidant reagent. After incubation at 37 °C for 2 h, 1 µl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in $H_2O$) was added to each tube and the mixed solution was loaded on 1% agarose gel. The electrophoresis was carried out for 1.5 h at 100 V in TBE buffer (89 mM Tris–borate, pH 8.3, 2.5 mmol $l^{-1}$ EDTA). Gels were stained with ethidium bromide (1 mg $ml^{-1}$) for 10 min prior to being photographed under UV light. Figure 8 gives the DNA cleavage results of the electrophoresis on agarose gel. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form linked circular (LC) or nicked circular (NC) DNA from its supercoiled (SC) form by quantitatively estimating the intensities of the bands using the Biolab UVItec gel documentation system. The fraction of each form of DNA was calculated by dividing the intensity of each band by the total intensities of all the bands in the lane.

## Results and Discussion

Modifications of formerly described syntheses of the Schiff base ligand[46–49] and homo- and hetero polynuclear copper(II) complexes[49] involving the Schiff base ligand and 1,10-phenanthroline were used to prepare the ligand and its complexes (**1–5**). The melting points, yields, colors, magnetic susceptibilities, molar conductivity values and elemental analyses of the prepared complexes and ligand are summarized in Table 1. All complexes are stable at room temperature. Single crystals of the compounds could not be isolated from any organic solution, thus no definitive structures can be described. However, the analytical, spectroscopic and magnetic data enables us to predict possible structures as depicted in Figs 1–4. The metal : L : phen ratio was found to be 2 : 1 : 2 for dinuclear complexes and the metal : L ratio was found to be 3 : 2 for trinuclear complex by elemental analyses.

### $^1$H- and $^{13}$C-NMR Spectra

*N*,*N*′′-bis[1-biphenyl-2-hydroxyimino-2-pyrrolidino-1-ethylidene]-diethylenetriamine ($H_2L$) in $CDCl_3$ was studied by $^1$H- and $^{13}$C-NMR spectroscopies. The deuterium-exchangeable proton of the (=N–OH) group for the $H_2L$ showed a chemical shift at 8.45 ppm as a singlet. The $^{13}$C-NMR spectral data of the ligand confirmed the results of the $^1$H-NMR spectra. In the $^{13}$C-NMR spectra of the ligand $H_2L$, the signal at 173.76 ppm was attributed to the azomethine C(6) atom which also confirmed the structure of ligand. The chemical shift for the C(1) atom of the oxime group was recorded at 145.12 ppm. The other observed chemical shifts are given in Experimental section. All of these values prove that the ligand formed and are in good agreement with the values previously reported.[49–52] Since all metal complexes are paramagnetic, their $^1$H- and $^{13}$C-NMR spectra could not be obtained.

**Table 1.** Physical properties and elemental analysis of the Schiff base ligand and its complexes

| Compd | Formula | m.p. (°C) | Yield (%) | Color | $\mu_{eff}$ (B.M.) | $\Lambda_M$[b] | Calcd (found) % C | H | N | Metal |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $[C_{40}H_{45}N_7O_2]$ | 63 | 45 | Brown | – | – | 73.26 (73.12) | 6.92 (6.86) | 14.95 (14.84) | – |
| **2** | $[C_{64}H_{61}N_{11}O_{11}Cu_2Cl_2]$ | [a]222 | 58 | Green | 1.81 | 185 | 56.60 (56.79) | 4.53 (4.45) | 11.34 (11.23) | Cu 9.36 (9.52) |
| **3** | $[C_{64}H_{61}N_{11}O_{11}CuMnCl_2]$ | >300 | 65 | Green | 3.47 | 165 | 56.96 (56.78) | 4.56 (4.69) | 11.42 (11.53) | Cu 4.71 (4.86) Mn 4.07 (4.36) |
| **4** | $[C_{64}H_{61}N_{11}O_{11}CuCoCl_2]$ | [a]242 | 59 | Green | 2.76 | 182 | 56.79 (56.57) | 4.54 (4.51) | 11.38 (11.25) | Cu 4.69 (4.73) Co 4.35 (4.41) |
| **5** | $[C_{80}H_{90}N_{14}O_{14}Cu_3Cl_2]$ | [a]220 | 52 | Green | 2.16 | 187 | 55.44 (55.21) | 5.23 (5.35) | 11.31 (11.52) | Cu 11.00 (11.26) |

[a] Decomposition point.
[b] Molar conductivity ($\Omega^{-1}$ $cm^2$ $mol^{-1}$).

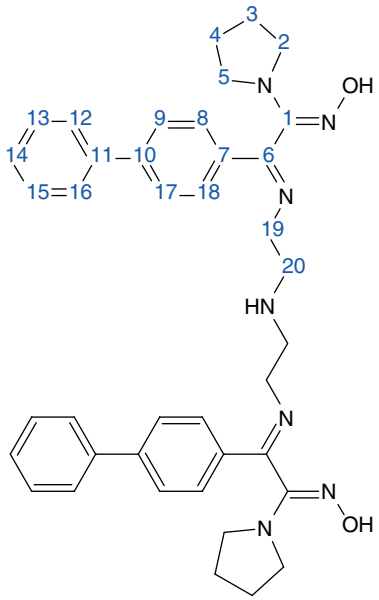

**Figure 1.** Structure of the ligand, $H_2L$ (**1**).

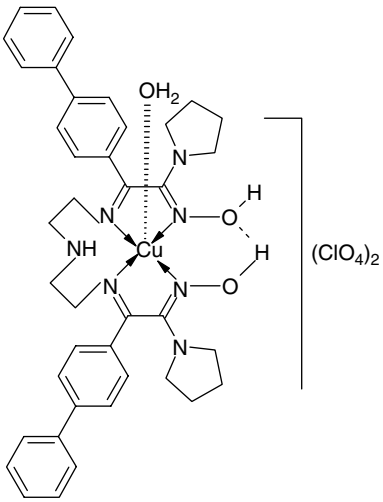

**Figure 2.** Proposed structure of the mononuclear copper(II) complex of $H_2L$.

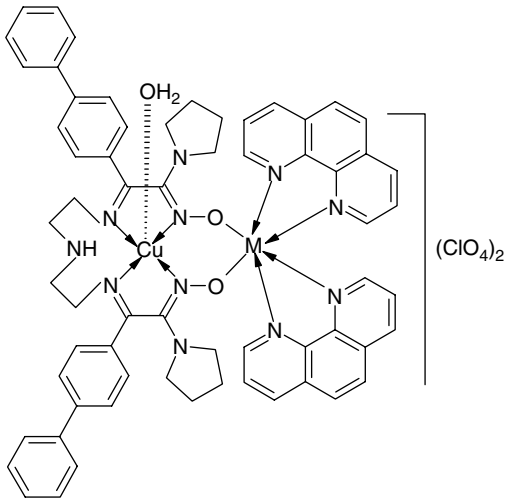

**Figure 3.** Proposed structure of the dinuclear Cu(II) complexes of $H_2L$, M=Cu(II) (**2**), M = Mn(II) (**3**), M = Co(II) (**4**).

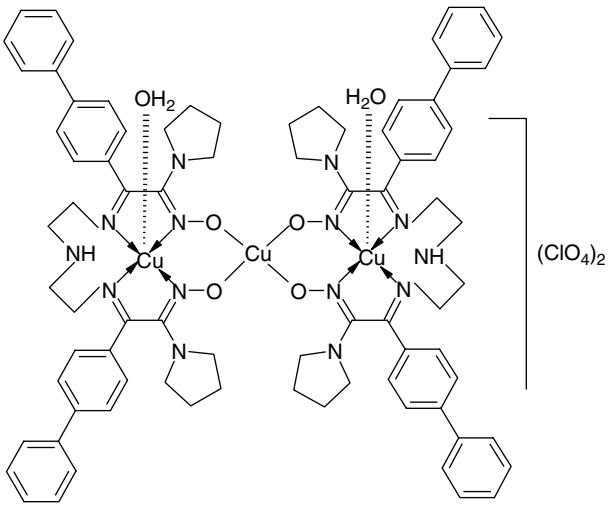

**Figure 4.** Proposed structure of the homotrinuclear Cu(II) (**5**) complex of $H_2L$.

### IR Spectra

The important IR spectral data of the free ligand and their homo- and heteropolynuclear copper(II) complexes are presented in Table 2. The characteristic vibrational frequencies have been identified by comparing the spectra of the complexes with their free ligand.

The spectra of $H_2L$ do not show absorptions characteristic of the C=O function owing to the formation of the hydrazone. The free ligand shows band at 3207 $cm^{-1}$ assigned to the O–H vibration of the oxime group. For the complexes, the strong O–H stretching bands at 3207 $cm^{-1}$ of the free ligand is absent in the spectra of the complexes, which indicates that the oxime oxygen atom is coordinated to the metal atom. In the free oxime, $\nu$(N–O) is observed at 1435 $cm^{-1}$. The shifts of these bands towards lower frequency by about 18–24 $cm^{-1}$ in the spectra of the complexes suggests participation of the oxime oxygen in coordination.[49] Coordination of the oxime and the imine nitrogen in the chelate ring is supported by the appearance of new bands at 418–432 and 507–516 $cm^{-1}$, which are assigned to $\nu$(M–N) and $\nu$(M–O), respectively.[53,54]

The medium band at 1670 $cm^{-1}$ is assigned to the $\nu$(C=N) stretching vibration of azomethine of the ligand. Coordination of the Schiff base to the metal ion through the azomethine nitrogen atom is expected to reduce the electron density in the azomethine link and, thus, to lower the C=N absorption frequency. Hence, this band undergoes a shift to lower frequency to 1635–1658 $cm^{-1}$ after complexation indicating the coordination of the azomethine nitrogen to Cu(II).[55] The copper(II) complexes exhibit medium intense broad bands centered at 3547–3582 $cm^{-1}$ assigned to coordinated water.[56–58] All of the perchlorate salts show a medium band near 1151–1182 $cm^{-1}$, a strong band at 1089–1095 $cm^{-1}$ (antisymmeric stretch) and a sharp band at 626 $cm^{-1}$ (antisymmetric bend), indicative of uncoordinated perchlorate anions.[57–59]

**Table 2.** Significant bands in the IR spectra of the Schiff base ligand and its complexes

| Compound | $\nu$(O–H) | $\nu$(N–H) | $\nu$(C=N)$_{im}$ | $\nu$(C=N)$_{ox}$ | $\nu$(N–O) | $\nu$(C–N) | $\nu$($ClO_4$) | $\nu$(M–O) | $\nu$(M–N) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 3207b | 3367m | 1670m | 1604s | 1435s | 1489m | – | – | – |
| **2** | 3547b | 3358w | 1658w | 1597s | 1423m | 1485w | 1095s, 1182w, 626w | 507w | 418w |
| **3** | 3566b | 3387m | 1649w | 1583w | 1427m | 1516m | 1091s, 1159m, 626w | 516w | 426w |
| **4** | 3582b | 3387w | 1649w | 1597m | 1423m | 1512m | 1089s, 1161w, 626w | 513w | 428w |
| **5** | 3556b | 3331w | 1635w | 1593s, | 1421s | 1519m | 1093s, 1151m, 626w | 507w | 432w |

s, strong; m, medium; w, weak; b, broad.

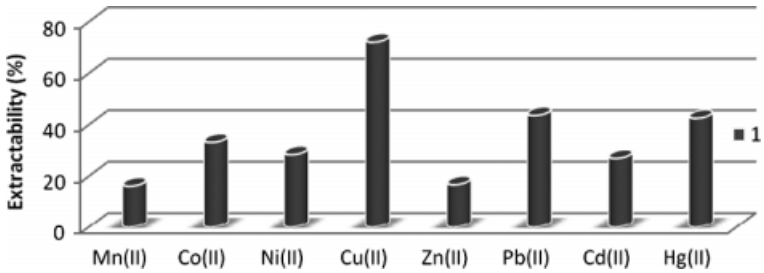


**Figure 5.** Extraction percentages of the metal picrates with ligand $H_2L$. $H_2O$–$CHCl_3$ = 1:1 (v/v): [picric acid] = $2 \times 10^{-5}$ M, [ligand] = $1 \times 10^{-3}$ M, [metal nitrate] = $1 \times 10^{-2}$ M, 298 K, 1 h contact time.

## Molar Conductance

Conductivity measurements of the complexes were determined using freshly prepared solutions of the complexes in *N*,*N*-dimethylformamide at room temperature. The molar conductivity values of the complexes are given in Table 1. The molar conductivities of the homo- and heteropolynuclear copper(II) complexes in *N*,*N*-dimethylformamide are in the range reported for 1 : 2 electrolytes in this solvent.[60]

## Magnetic Moment Studies

As is known, magnetic susceptibility measurements provide information regarding the structure of the complexes. Magnetic susceptibility was determined using a magnetic susceptibility balance. The room temperature effective magnetic moments of the complexes are given in Table 1. The observed room-temperature magnetic moment values for the homo- and heteronuclear copper(II) complexes are found to be paramagnetic.

Homodinuclear copper(II) complex (**2**) has magnetic moment value equal to 1.81 B.M. Furthermore the magnetic moments of the homotrinuclear copper(II) complex (**5**) at room temperature are found to 2.16 B.M. The magnetic moment values found for the homodi- and trinuclear copper(II) complexes are not consistent with the expected spin-only magnetic moment of an $S = 1/2$, Cu(II) $d^9$ system. In homodi- and homotrinuclear copper(II) complexes, the low values of the observed magnetic moments might be indicative of metal–metal interactions in the structure.

The magnetic moment value of the heterodinuclear copper(II)–manganese complex (3) is 3.47 B.M. while that of the heterodinuclear copper(II)–cobalt(II) complex (4) is 2.76 B.M. Magnetic data show that manganese(II) and cobalt(II), which are in an octahedral environment adopts a high-spin configuration in the heterodinuclear copper(II) complexes.[61]

As seen from Table 1 the homodi-, homotri- and heterodinuclear copper(II) complexes have subnormal magnetic moment values. The strong antiferromagnetic coupling that was found for the homo- and heteropolynuclear copper(II) complexes are explained by the good superexchange properties of the oximato or oxamidato groups. These results show that the axial coordination of perchlorate anion is not important to their magnetic behavior.[62]

## Solvent-extraction Studies

The extraction efficiency of the ligand $H_2L$ containing $N_4$ donor set toward transition metal ions [Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), Cd(II), Hg(II)] was determined by the picrate extraction method developed by Pedersen.[63] Extraction efficiency of the ligand was carried out by the two-phase solvent extraction of transition metal picrates into chloroform under neutral conditions. The results data have been obtained by using chloroform solution of these dimine–dioxime compounds to extract metal picrates from an aqueous phase. The equilibrium concentration of picrate in aqueous phase was then determined spectrophotometrically. The data are expressed as percentages of the cation extracted (E%) by the ligand (Fig. 5).

As can be seen in Fig. 5, the extractability of transition metal picrates differed in Cu(II) > Pb(II) > Hg(II) > Co(II) > Ni(II) > Cd(II) > Zn(II) > Mn(II) order and ligand (**1**) extracted all the selected metal cations between 16.18 and 72.15% when chloroform was used as organic solvent. Figure 5 shows that dimine–dioxime ligand $H_2L$ is a good extractant for the selected transition metal cations and this ligand showed the highest extraction ability for Cu(II) among all metal ions. This is an expected result because of the interaction of soft donor atom–soft metal cation. Ligand $H_2L$ has a nitrogen soft donor atom set. Therefore, it will interact with soft metal cations, such as Cu(II), between the tested metal cations. The cation binding properties of the ligand depend upon different factors such as macrocyclic effect, cavity size, the hard and soft acids and bases (HSAB) principles and the type and number of donor atoms. Also the precence of oxime groups (–C=N–OH) indicates that the oxime groups play an important role in the extraction process.[64] It is difficult to comment on whether or not the increase in extraction capability is the result of oxime groups

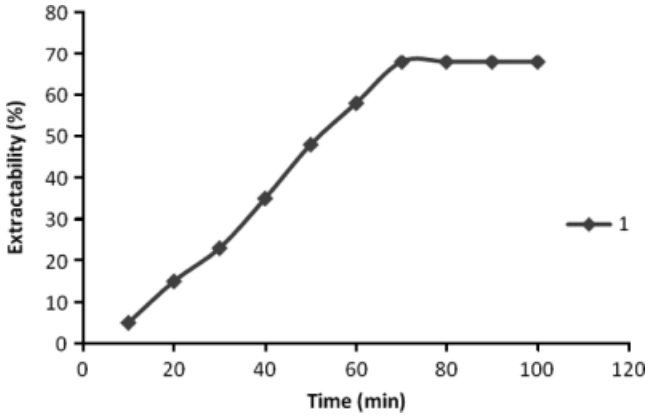


**Figure 6.** Effect of equilibrium time on the extraction of metal picrate. $H_2O$–$CHCl_3$ = 1:1 (v/v): [picric acid] = $2 \times 10^{-5}$ M, [ligand] = $1 \times 10^{-3}$ M, [metal nitrate] = $1 \times 10^{-2}$ M, 298 K, 1 h contact time.

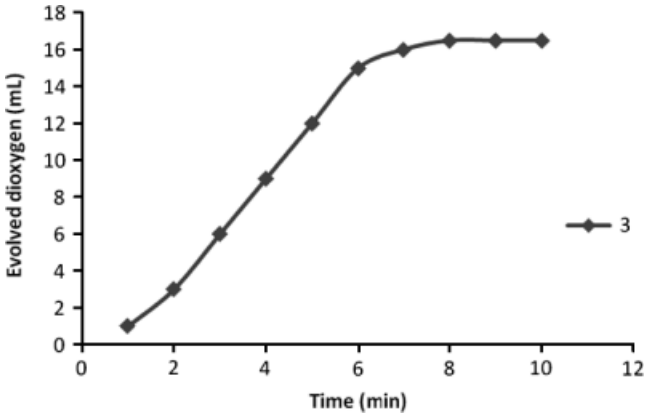


**Figure 7.** Time courses of dioxygen evolution in the disproportionation of $H_2O_2$ by complex **3** with added base imidazole in DMF. [complex] = 0.005 mmol, [$H_2O_2$] = 1.33 mmol, [imidazole] = 50 mg, 298 K.

or the increase in the number of donor atoms, but, according to these data, we can conclude that the hard and soft acids and bases principles and the number of donor atoms are much more effective than the other factors.[34,65] From these results, we could conclude that compound **1** is an excellent extractor for Cu(II).

In this study we also investigated the effect of contact time for the extraction process. To study the influence of equilibration time on the extraction of Cu(II), we placed an aqueous solution of metal picrate ($2 \times 10^{-5}$ M, 10 ml) in contact with an organic phase containing ligand ($1 \times 10^{-3}$ M, 10 ml) for 10–100 min. The results showed that the ligand **1** underwent a maximum extraction for Cu(II) after shaking for 60 min or longer. However, once the maximum extraction had been reached, increasing the contact time had no significant effect on the extraction. Therefore, an equilibrium time of 60 min was adopted for subsequent extraction studies to ensure complete extraction (Fig. 6).

## Catalase-like Function

All the complexes display catalytic ability for the disproportionation of $H_2O_2$ but significantly, the activity of complex **3** is relatively higher than the other complexes. For this reason the reactivity of this complex toward $H_2O_2$ is given in this study. The catalase-like function of the heterodinuclear copper(II)–manganese(II) complex (**3**) to disproportionate $H_2O_2$ into $H_2O$ and $O_2$ was examined at room temperature by volumetric measurements of evolved dioxygen. Unfortunately, copper(II) complexes of diimiine–dioxime ligand are all insoluble in water; therefore, catalytic activities of the complexes were determined in *N*,*N*-dimethylformamide. The reactivity studies indicated that the complex itself was catalytically almost inactive, but the decomposition of $H_2O_2$ was enhanced in the presence of a base such as 1-methylimidazole (1-MeimH), imidazole (imH) or pyridine (py) because of their strong $\pi$-donating ability. The evolution profile in Fig. 7 shows the involvement of a fast catalytic process occurring at the initial stage followed by a short slow period process to finish the reaction. It was suggested that these bases may be essential in the catalysis disproportionation of $H_2O_2$ by manganese catalase since they are known to be present in the vicinity of active sites of catalase and other manganoenzymes.[66] Furthermore the presence of the bidentate chelating nitrogen donor ligand phenanthroline in the coordination sphere of the metal significantly enhances the ability of the manganese to disproportionate $H_2O_2$, and the phenanthroline (phen) and 2,2′-bipyridine (bipy) species were found to be the more aggressive peroxide disproportionation catalysts.[67]

In the catalytic process the electron transfer only occurs between the Cu(II)–Cu(II) ions in the dimer structure. However when the second coordinate metal ion was Mn(II), the reactivity was greatly enhanced. The main reason is that $Mn_2$(II) complexes are also good candidates for $H_2O_2$ dismutation. Gao and co-workers supposed that the inter-molecular Mn(II)–Mn(II), Cu(II)–Mn(II) and Cu(II)–Cu(II) coupling are possibly active centers for $H_2O_2$ dismutation in the CuMn system.[45]

## DNA Interactions

The interactions of the Schiff base ligand (**1**), its homodinuclear copper(II) (**2**), heterodinuclear copper(II)–manganese(II) (**3**), heterodinuclear copper(II)–cobalt(II) (**4**) and homotrinuclear copper(II) (**5**) complexes with DNA in the absence or presence of $H_2O_2$ as a cooxidant were electrophoretically investigated using the supercoiled form of pBR 322 DNA. DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (form I) to nicked circular DNA (form II) and linear DNA (form III) under aerobic conditions. When circular plasmid DNA is conducted by electrophoresis, the fastest migration is 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. Control experiments were applied using only DNA and DNA + $H_2O_2$. As shown in Fig. 8, incubation of the pBR 322 DNA at 37 °C for 2 h with 1 μg of the compounds caused the conversion of form I to form II. The cleavage efficiency after incubation for 2 h in the absence of $H_2O_2$ follows the order: **2** > **4** > **3** > **5** > **1**. These results indicate that the examined complexes induces very similar conformational changes on supercoiled DNA and these changes occurred in a sequential manner involving conversation of supercoiled form to nicked form and then the linear form, but **3** and **5** are less effective than complexes **2** and **4**. On the other hand, the pBR 322 DNA treated with the ligand **1** showed only insignificant changes in the form levels compared with the control DNA. Namely, the ligand **1** alone is cleavage-inactive. In the presence of $H_2O_2$ as a cooxidant, the probability of double-strand scissions is enhanced once the DNA has undergone a single strand break. It is clear that the degradation of pBR 322 DNA is dependent on cooxidant used. This is displayed in the gel by the appearance

  

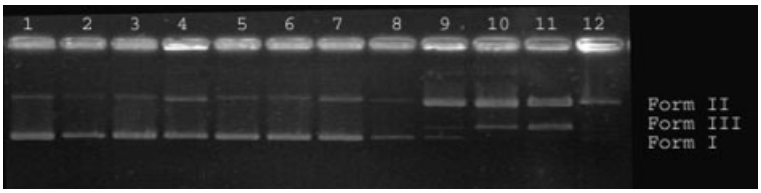


**Figure 8.** Gel electrophoresis diagram showing the cleavage data of pBR322 plasmid DNA (0.1 μg) by the ligand and its complexes in DMF–Tris buffer medium (pH 8.0) in air after incubation at 37 °C for 2 h. Lane 1, untreated pBR322 plasmid DNA; lane 2, pBR322 plasmid DNA + $H_2O_2$; lanes 3–7, pBR322 plasmid DNA + the compounds = **1**–**5**, respectively; lanes 8–12, pBR322 plasmid DNA + the compounds + $H_2O_2$ (the compounds = **1**–**5**, respectively).

**Table 3.** DNA cleavage data of pBR322 plasmid DNA (0.1 μg) by **1**–**5**

| Lane no | Reaction conditions | Incubation time (h) | Form I %SC | Form II %NC | Form III %LC |
|---|---|---|---|---|---|
| 1 | DNA | 2 | 69.4 | 30.6 | ND |
| 2 | DNA + $H_2O_2$ | 2 | 65.4 | 34.6 | ND |
| 3 | DNA + **1** | 2 | 69.8 | 30.2 | ND |
| 4 | DNA + **2** | 2 | 49.0 | 51.0 | ND |
| 5 | DNA + **3** | 2 | 64.7 | 35.3 | ND |
| 6 | DNA + **4** | 2 | 63.0 | 37.0 | ND |
| 7 | DNA + **5** | 2 | 65.5 | 34.5 | ND |
| 8 | DNA + **1** + $H_2O_2$ | 2 | 64.7 | 35.3 | ND |
| 9 | DNA + **2** + $H_2O_2$ | 2 | 32 | 58 | 10 |
| 10 | DNA + **3** + $H_2O_2$ | 2 | ND | 61.3 | 38.7 |
| 11 | DNA + **4** + $H_2O_2$ | 2 | ND | 74.6 | 25.4 |
| 12 | DNA + **5** + $H_2O_2$ | 2 | ND | 100 | ND |

SC, NC, LC, supercoiled, nicked circular and linked circular forms of DNA, respectively.
ND, not detected.

of linear DNA molecules (form III), as shown in Fig. 8 (lanes 9–11). The percentage of linear DNA molecules in the presence of $H_2O_2$ follows the order: **3** > **4** > **2**. The cleavage percentages are listed in Table 3. From these results, we found that the complexes **2**–**4** can cleave the supercoiled DNA to nicked and linear DNA with cooxidant $H_2O_2$. In the presence of cooxidant $H_2O_2$, the intensity of the circular supercoiled DNA (form I) band was found to decrease, while that of nicked (form II) and linear DNA (form III) bands apparently increases. After incubation of the pBR 322 DNA for 2 h with the complexes **3**–**5**, the circular supercoiled DNA (form I) band disappeared completely (Fig. 8, lanes 10–12). These results are similar to that observed for some Cu(II) and Co(II) complexes used as chemical nucleases.[22,23,26] Further studies are underway to clarify the cleavage mechanism.

In addition, the chemical environment around the central metal ions and their geometric structures may also affect the nucleolytic efficiency of the complexes.[29,31] Therefore, the difference in the DNA cleavage activities of the Schiff base complexes may be attributed to their proximity to the DNA on binding since the phenanthroline units present in **2, 3** and **4** may provide much more effective binding than **5**, which has no such structural units. This may also imply that the binding of **2, 3** or **4** to DNA makes metal ions more approachable to the DNA backbone than those in **5**. Therefore, the difference in the cleavage behavior of **5** is consistent with a distinct oxidative cleavage pathway. These observations suggest that the coordination environment of the central metal ions in the complexes not only governs DNA binding but also determines the nucleolytic action.[29]

## Conclusions

A series of Cu(II) complexes (**2**–**5**) derived from a new tetradentate Schiff base ligand (**1**) and and 1,10-phenanthroline have been synthesized and characterized using different spectroscopic techniques. From the elemental analyses, stoichiometric and spectroscopic studies discussed above, the ligand has been shown to act as a tetradentate which coordinates through the nitrogen atoms of the oxime and imine groups. In the dinuclear complexes, in which the first copper(II) ion was complexed with nitrogen atoms of the oxime and imine groups in a square pyramidal coordination geometry, the second copper(II) ion is ligated with dianionic oxygen atoms of the oxime groups and linked to the 1,10-phenanthroline nitrogen atoms. However, the trinuclear Cu(II) complex was formed by the coordination of the third Cu(II) ions with dianionic oxygen atoms of each of the two molecules of the mononuclear copper(II) complexes. The suggested structures of these complexes are shown in Figs 2–4. The solvent extraction of various transition metal cations from the aqueous phase to the organic phase was carried out by using dimine–dioxime ligand (**1**). The extractability of transition metal picrates [Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), Cd(II), Hg(II)] was evaluated. It was found that the ligand had strong affinity towards Cu(II) ion and that could be used as an effective reagent for the extraction of Cu(II) ion from aqueous solutions. Furthermore in the catalytic study, heterodinuclear copper(II)–manganese(II) complex (**3**) exhibited high activity for catalyzing disproportionation of $H_2O_2$ to $H_2O$ and $O_2$. It was proposed that any residual hydrogen peroxide formed during industrial processes can be decomposed prior to disposal through the application of immobilized transition metal complexes. The DNA cleavage results showed that the homo- and heterodinuclear copper complexes can effectively cleave supercoiled DNA to form nicked or linear DNA by performing single strand and double strand scissions under aerobic conditions in the presence of hydrogen peroxide as co-oxidant.

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