

Proceedings of the 50th Zakopane School of Physics, Zakopane, Poland, May 18–23, 2015

Synthesis, Characterization of Novel Dinuclear Co(II)–Cu(II) Complexes and Investigation of Their Catecholase and Catalase-Like Activities

B. DEDE^{a,*}, E. CICEK^b AND F. KARIPCIN^c^aFaculty of Science and Art, Süleyman Demirel University, Isparta, Turkey^bFaculty of Science and Art, Mehmet Akif Ersoy University, Burdur, Turkey^cFaculty of Science and Art, Nevşehir Hacı Bektaş Veli University, Nevşehir, Turkey

The purpose of this study is synthesis, characterization and enzyme activity functions of novel dinuclear metal complexes. Therefore two new dinuclear heterometallic cobalt(II)–copper(II) complexes have been synthesized and structurally characterized by using Fourier transform infrared method, elemental analysis, inductively coupled plasma optical emission spectrometry, molar conductivity, magnetic moment measurements and thermal analysis. Spectroscopic and stoichiometric data of the metal complexes indicated that the metal:ligand ratio of the complexes were found to be 2:1. Both of the complexes are 1:2 electrolytes as shown by their molar conductivities and paramagnetism. The subnormal magnetic moment values of the dinuclear complexes were explained by an antiferromagnetic interaction. Additionally complexes were each tested both for their ability to oxidation reaction of 3,5-di-*tert*-butylcatechol to the 3,5-di-*tert*-butyl-*o*-benzoquinone presence of O₂ and catalyse the disproportionation of hydrogen peroxide in the presence of the added base imidazole. It was found that both of the complexes exhibited good catecholase and catalase-like enzyme activity.

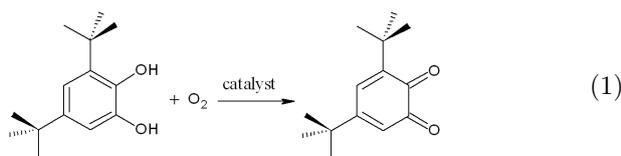
DOI: [10.12693/APhysPolA.129.203](https://doi.org/10.12693/APhysPolA.129.203)

PACS: 81.20.Ka, 82.80.–d, 87.15.R–

1. Introduction

The chemistry of complexes containing mixed-donor macrocyclic ligands with cation complexing abilities has been explored extensively in the last few decades [1]. Some metal complexes have been used extensively for various purposes and also have received considerable attention as model compounds which mimic biofunctions such as the catecholase and catalase enzyme activity [2, 3].

Catechol metabolism is important both biologically and environmentally. Catechol oxidase is often inferred to be involved in plant defense as highly reactive *o*-quinones autopolymerize to brown polyphenolic catechol melanins, a process that is thought to protect the damaged plant from pathogens or insects (Eq. (1)) [4].



Catalase, one of the three major sensitive protective enzymes in living organisms, exists in almost all aerobically respiring organisms. It dismutates H₂O₂ according to Eq. (2) and protects cells from the toxic effects of hydrogen peroxide (H₂O₂), the latter being linked to a variety of pathological consequences such as aging, diabetes and cancer [5–7]



The present work describes the synthesis and characterization of novel heterodinuclear Co(II)–Cu(II) derived from iminoxime ligands. Catecholase and catalase-like activities of the complexes are also studied.

2. Experimental

All chemicals used were of high purity grade and were used without further purification. Biphenyl, AlCl₃, chloroacetyl chloride, isopentyl nitrite, pyrrolidine, *p*-toluidine, 1,3-propanediamine, acetone, triethylamine, Co(OAc)₂·4H₂O, Cu(ClO₄)₂·6H₂O, 1,10-phenanthroline monohydrate, HCl, chloroform, CaCl₂, 3,5-di-*tert*-butylcatechol, H₂O₂ (30%, w/w), dimethylformamide, imidazole, ethyl alcohol, methyl alcohol, dichloromethane, H₂SO₄, NaCl, diethylether, P₄O₁₀, hexane and NaHCO₃ were used in synthesis and enzyme activity studies of the metal complexes.

2.1. Physical measurements

Elemental analyses (C, H, N) were performed using a LECO 932 CHNS analyzer and metal contents were obtained on a Perkin Elmer Optima 5300 DV ICP-OES Spectrometer. IR spectra were recorded using KBr discs (4000–400 cm^{–1}) on a Shimadzu IRPrestige-21 FT-IR Spectrophotometer. The UV/VIS measurements were carried out on a PG T80+ UV/VIS spectrophotometer using matched 1.0 cm quartz cells. Molar conductivity values of the complexes were determined in DMF at ambient temperature using a Optic Ivymen System conductivity meter. Thermal analyses of the complexes were performed in atmospheric air using a Perkin Elmer Diamond TGA thermal analyzer and operating at 10 °C/min. Melting points were determined on an

*corresponding author; e-mail: bulentdede@sdu.edu.tr

Electrothermal model IA 9100. Room temperature magnetic susceptibility measurements were done on a Sherwood Scientific Magnetic Susceptibility Balance (Model MX1) by the Faraday method using the magnetic susceptibility standard HgCo(NCS).

2.2. Synthesis of complexes

Caution: Perchlorate salts of metal complexes are potentially explosive and should be handled in small quantities.

Ligands N, N' -bis[1-biphenyl-2-hydroxyimino-2-(pyrrolidino)-1-ethylidene]-1,3-propanediamine (H_2L^1) and N, N' -bis[1-biphenyl-2-hydroxyimino-2-(4-methylanylino)-1-ethylidene]-1,3-propanediamine (H_2L^2) were prepared according to previously published procedure [8].

A solution of $Co(OAc)_2 \cdot 4H_2O$ (1 mmol) in acetone (25 mL) was added to the ligand solution (1 mmol) in 30 mL acetone, 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 cobalt(II) complexes were used without further purification.

The mononuclear cobalt(II) complex (1 mmol) was mixed with Et_3N (1 mmol) in MeOH (20 mL) and stirred for 0.5 h. The solutions of $Cu(ClO_4)_2 \cdot 6H_2O$ (1 mmol) in MeOH (10 mL) and 1,10-phenanthroline monohydrate (2 mmol) in MeOH (10 mL) were successively added to the resulting solution 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} .

2.3. Catecholase activity

The catalytic oxidation of the 3,5-di-*tert*-butylcatechol (3,5-DTBC) by the complexes was monitored spectrophotometrically recording the increase in absorbance

at 395 nm, corresponding to the formation of the *o*-quinone product 3,5-di-*tert*-butyl-*o*-benzoquinone (3,5-DTBQ). The observed rate constant, k_{obs} values of the complexes for the *o*-quinone formation, were also obtained from Eq. (3):

$$\log(A_\infty/A_\infty - A_t) = kt. \quad (3)$$

A_∞ and A_t are the absorbance of the formed 3,5-di-*tert*-butyl-*o*-benzoquinone at time = ∞ and t , respectively.

2.4. Catalase-like activity

Volumetric measurements of evolved dioxygen during the reactions of the dinuclear complex with H_2O_2 were studied as follows: A 50 mL three-necked round-bottom flask containing a solution of the metal complex (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 volumetry. 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 metal complexes were either inactive or very weak catalysts for this reaction).

3. Results and discussion

The heterodinuclear Co(II)–Cu(II) complexes have been prepared by reaction of appropriate metal salts with the ligands including oxime and imine group. The physical and analytical data of the complexes are presented in Table I and Table II. These data are in good agreement with the proposed iminoxime metal complexes (1) and (2) whose structures are given in Fig. 1.

Physical properties of the metal complexes.

TABLE I

compound	μ_{eff} [μ_B]	Λ_M [$\Omega^{-1} cm^2 mol^{-1}$]	color	m.p. [°C]	yield [%]
(1) $[Co(L^1)(H_2O)_2Cu(phen)_2](ClO_4)_2$	2.76±0.07	165±6	brown	226±1	76±5
(2) $[Co(L^2)(H_2O)_2Cu(phen)_2](ClO_4)_2$	2.43±0.05	172±7	brown	264±1	65±4

Elemental analysis of the metal complexes.

TABLE II

compound	calculated (found) [%]				
	C	H	N	Co	Cu
(1)	56.36 (56.14±0.27)	4.50 (4.62±0.14)	10.43 (10.18±0.22)	4.39 (4.57±0.07)	4.73 (4.91±0.14)
(2)	58.58 (58.36±0.21)	4.27 (4.43±0.08)	9.90 (9.72±0.18)	4.17 (4.48±0.16)	4.49 (4.64±0.06)

The important IR spectral bands for the synthesized complexes are given in Table III. The broad $\nu(OH)$ bands at 3201 and 3264 cm^{-1} observed in the IR spectra of

the ligands H_2L^1 and H_2L^2 are absent in the IR spectra of their dinuclear Co(II)–Cu(II) complexes indicating deprotonation of the OH groups and formation of bond

Significant bands in the IR spectra of the metal complexes (cm^{-1}).

TABLE III

Compounds	O–H	N–H	C=N	C=O	N–O	C–N	ClO_4	M–O	M–N
$(\text{H}_2\text{L}^1)^a$	3201b	–	1660w	1606s	1450s	1483m	–	–	–
$(\text{H}_2\text{L}^2)^a$	3264b	3377m	1667s	1601s	1375m	1517m	–	–	–
(1)	3596b	–	1647w	1594m	1423m	1496m	1094s, 1161m, 626w	512w	418w
(2)	3571b	3394w	1641w	1589m	1362m	1526s	1102s, 1163w, 625w	517w	426w

(b: broad, s: strong, m: medium, w: weak); ^aRef. [8].

between metal and oxygen. This is supported by the appearance of a new band at about 515 cm^{-1} attributed to (M–O) stretching and shifting $\nu(\text{NO})$ to the lower frequency. Broad peaks appearing at 3596 and 3571 cm^{-1} in the spectra of the metal complexes indicate that water molecule is coordinated to the metal(II) for the (1) and (2), respectively. The ligands show characteristic band at about 1660 cm^{-1} assigned to stretching vibration of imine group, however this band is lowering in the frequency (13 and 26 cm^{-1}) when the complex formation takes place, indicating coordination through the imine nitrogen. This idea is supported by the appearance of a new band at 418 and 426 cm^{-1} assigned to $\nu(\text{M–N})$ for the (1) and (2), respectively. The synthesized complexes show also bands at about 1100 , 1160 and 625 cm^{-1} . These features are typical for uncoordinated perchlorates. The FT-IR data of the synthesized metal complexes are in good agreement with those of known iminoxime complexes [8–10].

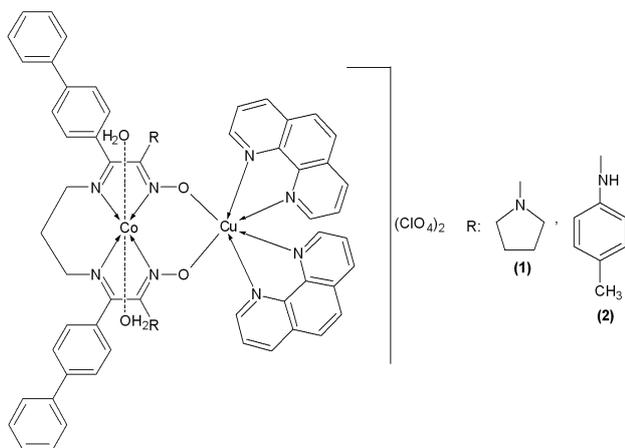


Fig. 1. Proposed structure for the complex (1) and (2).

Magnetic susceptibility was determined using a magnetic susceptibility balance. The magnetic moment values of the metal complexes at room temperature are reported in Table I and both complexes also found that paramagnetic nature. But these magnetic moment values are less than expected. The low values of magnetic moment (2.76 and 2.43 BM) of the complexes (1) and (2) are indicative of antiferromagnetic exchange [11, 12].

Thermal analyses of the complexes have been performed using thermogravimetry-differential thermogravimetry (TG-DTG) techniques. The thermogravimet-

ric analyses for the synthesized complexes were carried out within the temperature range from room temperature up to 900°C . It was found that the thermal behaviour of all the complexes was similar. The thermal decomposition of the complexes proceeds with three main degradation steps. The first step occurs within the temperature about 130°C which is reasonably accounted for the loss of two coordinated water. The second mass loss at 130 – 460°C is due to the liberation of two ClO_4^- and two phenanthroline groups. The third decomposition starts at 460°C corresponding to loss of two biphenyl groups. The last step did not finish completely at 900°C .

Molar conductivity measurements of the metal complexes were determined using freshly prepared solutions of the complexes in DMF at room temperature. The molar conductivity values of the complexes are given in Table I. The molar conductivities of the heterodinuclear Co(II)–Cu(II) are 165 and $172 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ and these values are in the range reported for 1:2 electrolytes in this solvent [13].

3.1. Enzyme activity studies

The catecholase activities of the complexes (1) and (2) have been examined in methanol because of the good solubility of the complexes as well as of the substrate and of its product. The catalytic oxidation reaction does not proceed in the absence of the catalysts, the Co(II)–Cu(II) complexes (1) and (2).

Both of the complexes show significant catalytic activities toward the aerobic oxidation of the model substrate 3,5-di-*tert*-butylcatechol to the 3,5-di-*tert*-butyl-*o*-quinone as unique oxidation product (Fig. 2 and Fig. 3).

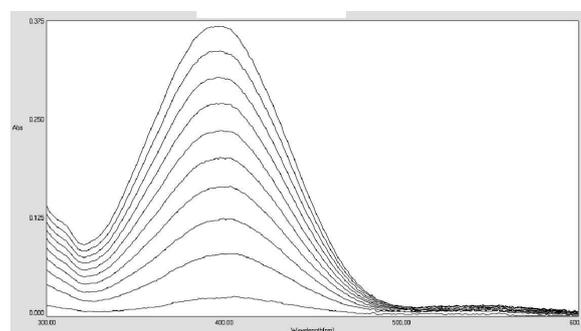


Fig. 2. The increasing density of absorption spectra at 395 nm of the (1) at 25°C (the spectra have been recorded at every 3 min interval).

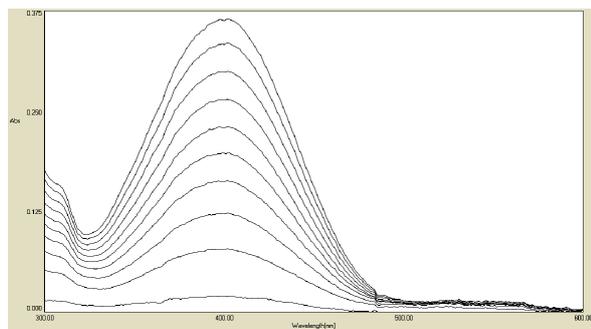


Fig. 3. The increasing density of absorption spectra at 395 nm of the (2) at 25 °C (the spectra have been recorded at every 3 min interval).

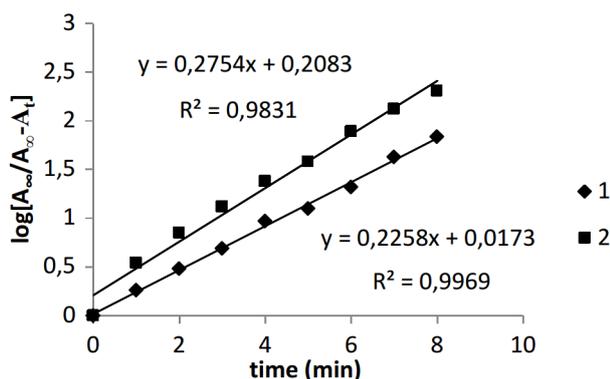


Fig. 4. The course of absorption maxima at 395 nm with time.

The kinetics of the oxidation reaction of 3,5-DTBC was determined by the method of initial rates by monitoring the growth of the 395 nm band of 3,5-DTBQ as a function of time. The catecholase activities of the metal complexes were obtained using a plot of $\log(A_{\infty}/(A_{\infty} - A_t))$ versus time and the rate constants of the complexes for the catalytic oxidation were given in Fig. 4. The rate constant values of the complexes show that the reactiv-

ity of complexes is differing from each other. As can be seen from the rate constant, complex (2) exhibited better catecholase activity than complex (1).

The catalase-like enzyme activities of the synthesized complexes were also studied. The catalytic activity of the complexes (1) and (2) towards the disproportionation of hydrogen peroxide was investigated by measuring the volume of evolved oxygen during the course of the reaction. When the complexes are used alone catalytic activity does not take place. This phenomenon was previously shown that the catalase mimetic activity of the manganese complexes is significantly improved in the presence of the base imidazole [14].

The time course of the O₂ evolution is shown in Fig. 5. As a result of the catalase-like activity studies, the complex (2) has high disproportionation efficiency when compared to the other synthesized complex (1). The complex (2) appears to be the most efficient catalyst in the first minute with 47 molecules of peroxide disproportionated by one molecule of the complex, when Table IV is examined. A comparison of the total number of molecules of H₂O₂ disproportionated by one molecule of the metal complex shows that complex (2) is the most effective catalyst with 276 molecules.

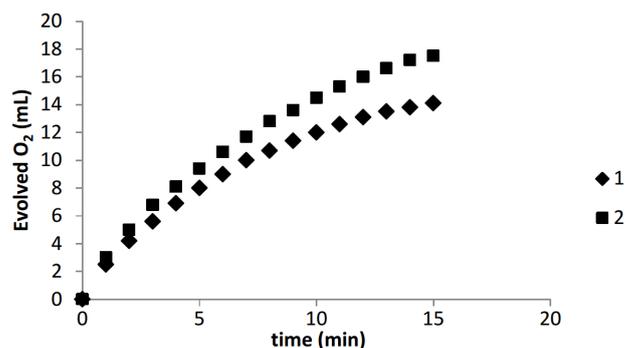


Fig. 5. Time course of oxygen evolution in the disproportionation of H₂O₂ by complex (1) and (2).

TABLE IV
Time course of oxygen evolution in molecules of H₂O₂ disproportionated by the complexes (1) and (2) at 25 °C.

Time cmps	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
(1)	39	66	88	109	126	142	158	169	180	189	198	206	213	217	222
(2)	47	79	107	128	148	167	184	202	214	229	241	252	262	271	276

1,10-phenanthroline has been used as the compartmental ligand in synthesis of the complexes. The presence of the bidentate chelating nitrogen donor ligand in the coordination sphere of the metal significantly enhances

the ability of the metals to disproportionate H₂O₂ and the phenanthroline (phen) and 2,2'-bipyridine (bipy) species were found to be the more aggressive peroxide disproportionation catalysts [15, 16].

4. Conclusion

Spectroscopic and analytical data presented above suggest that the complexes can be formulated as $[\text{Co}(\text{L}^1)(\text{H}_2\text{O})_2\text{Cu}(\text{phen})_2](\text{ClO}_4)_2$ and $[\text{Co}(\text{L}^2)(\text{H}_2\text{O})_2\text{Cu}(\text{phen})_2](\text{ClO}_4)_2$. Moreover, in heterodinuclear complexes the first Co(II) ion was coordinated with nitrogen atoms of the oxime and imine groups, the second ion [Cu(II)] is ligated with dianionic oxygen atoms of the oxime groups and are linked to the 1,10-phenanthroline nitrogen atoms. Dinuclear complexes of the iminoxime ligands have a 2:1 metal:ligand ratio.

Catecholase and catalase enzymes having important biological functions in organisms contain dinuclear metal sites. Therefore, dinuclear metal complexes are examined usually for this type of enzyme activity studies. The results of the studies in the literature show that dinuclear metal complexes have better catecholase and catalase enzyme activity compared to those with mononuclear metal complexes. Therefore, especially dinuclear Co(II)–Cu(II) complexes were synthesized in this study. Catalytic activity studies show that both of the complexes exhibited the activity similar to catecholase and catalase and represent the complexes (1) and (2) which can be regarded as structural models for a catecholase and catalase enzyme.

References

- [1] J.S. Bradshaw, E.K. Krakowiak, R.M. Izatt, *Aza-Crown Macrocycles*, Wiley, New York 1993 and reference therein; *Coordination Chemistry of Macrocyclic Compounds*, Ed. G.A. Melson, Plenum Press, New York 1979.
- [2] J. Kaizer, G. Barath, R. Csonka, G. Speier, L. Korecz, A. Rockenbauer, L. Parkanyi, *J. Inorg. Biochem.* **102**, 773 (2008).
- [3] F. Zippel, F. Ahlers, R. Werner, W. Haase, H.F. Noltling, B. Krebs, *Inorg. Chem.* **35**, 3409 (1996).
- [4] M.U. Triller, D. Pursche, W.Y. Hsieh, V.L. Pecoraro, A. Rompel, B. Krebs, *Inorg. Chem.* **42**, 6274 (2003).
- [5] M. Zamocky, F. Koller, *Prog. Biophys. Mol. Biol.* **72**, 19 (1999).
- [6] X. Chen, H. Xie, J. Kong, J. Deng, *Biosens. Bioelectron.* **16**, 115 (2001).
- [7] I. Fridovich, *Annu. Rev. Biochem.* **64**, 97 (1995).
- [8] B. Dede, F. Karipcin, M. Cengiz, *J. Hazard. Mater.* **163**, 1148 (2009).
- [9] A.E.M., Ramadan, İ.M. El-Mehasseb, R.M. Issa, *Transit. Met. Chem.* **22**, 529 (1997).
- [10] R. Blinc, D. Hadzi, *J. Chem. Soc.* **1958**, 4536 (1958).
- [11] R.N. Patel, N. Singh, V.L.N. Gundla, U.K. Chauhan, *Spectrochim. Acta Part A* **66**, 726 (2007).
- [12] R. Ruiz, F. Lloret, M. Julve, J. Faus, M.C. Munoz, X. Solans, *Inorg. Chim. Acta* **213**, 261 (1993).
- [13] W.J. Geary, *Coord. Chem. Rev.* **7**, 81 (1971).
- [14] M. Devereux, M. McCann, V. Leon, V. McKee, R.J. Ball, *Polyhedron* **21**, 1063 (2002).
- [15] M. McCann, M.T. Casey, M. Devereux, M. Curran, V. McKee, *Polyhedron* **16**, 2741 (1997).
- [16] M. McCann, M.T. Casey, M. Devereux, M. Curran, G. Ferguson, *Polyhedron* **16**, 2547 (1997).