J. Chil. Chem. Soc., 63, Nº 3 (2018)

CATALASE, CATECHOLASE AND PHENOXAZINONE SYNTHASE-LIKE ACTIVITIES OF HOMODINUCLEAR Co(II), Ni(II), Cu(II) and Zn(II) COMPLEXES INCLUDING OXIME GROUP

GÜVENÇ GÖRGÜLÜ[a], *BÜLENT DEDE*[b*]

[a]*Department of Science Education, Faculty of Education, Mehmet Akif Ersoy University, Burdur, Turkey*
[b*]*Department of Chemistry, Faculty of Science & Art, Süleyman Demirel University, Isparta, Turkey*

ABSTRACT

Homodinuclear (1) Co(II), (2) Ni(II), (3) Cu(II) and (4) Zn(II) metal complexes containing oxime group were tested for their catalase, catecholase and phenoxazinone synthase-like activity by volumetric and spectrophotometric procedures. Catalase-like activity of the complexes was studied by measuring the evolved dioxygen resulted from the disproportionation reaction of potentially harmful hydrogen peroxide. Catecholase and phenoxazinone synthase-like enzyme activities were followed by the increase in absorbance at 400 and 433 nm resulted from the oxidation reaction of 3,5-di-*tert*-butylcatechol to 3,5-di-*tert*-butylquinone and 2-aminophenol to 2-aminophenoxazine-3-one, respectively. Among the studied homodinuclear complexes, complex (3) showed the highest catalytic efficiency for three enzymes tested. Catalytic efficiency of the complexes was found 3>1>4=2 for catalase-like, 3>1>2>4 for catecholase-like and 3>1>4>2 for phenoxazinone synthase-like activity. Relatively higher catalytic activity of the Cu(II) complex is thought to be related to the lower redox potential of Cu(II) ion and better proximity of the chosen substrates with the complex (3).

Keywords: Homodinuclear metal complexes, oxidoreductases, catalase, catecholase, phenoxazinone synthase

1. INTRODUCTION

Metal ions have been found to play a crucial role in many biological systems. At least one-third of all proteins appear to contain metal ions and even the nucleozymes such as ribozymes (RNA enzymes) appear to be metalloenzymes.[1,2] These metal ions can modify electron flow in a substrate or enzyme, thus effectively controlling an enzyme-catalyzed reaction. Without the appropriate metal ion, a biochemical reaction catalyzed by a particular metalloenzyme would proceed very slowly. Bio-inorganic chemistry comes into the play at this point which encompasses a variety of disciplines, ranging from inorganic chemistry and biochemistry to spectroscopy, molecular biology, and medicine.[3] It is not possible to use the natural enzyme as a drug due to its delivery problems and instability in solution. Therefore, synthetic compounds able to mimic natural enzymes have been designed and studied by the researchers. [4-8]

Organic chelating ligands containing the –C=N–OH group have been named as oxime compounds.[9] Oxime ligands have a growing interest due to their physical, chemical and biological properties, besides their reactivity patterns. They find a broadening application field in biological systems,[10,11] medicinal chemistry,[12,13] electrochemical and electrooptical sensors.[14]

Several types of ligand systems, which can bind two metal ions in close proximity, have been used as biomimetic studies of binuclear metalloenzyme and metalloproteins due to their interesting catalytic properties, their ability to stabilize unusual oxidation states and possibilities for magnetic interaction between two metal ions.[15,16] These complexes have found many applications as catalysts for specific purposes, as mimics for metallobiomolecules, and in investigations concerning the mutual influence of two or more metal centers on the electronic, magnetic and electrochemical properties of such closely spaced metal centers.[17,18]

Oxidoreductases are a broad group of enzymes that catalyze the oxidation and reduction reactions. They involve various life-sustaining reactions ranging from the simplest organism to human beings. Catalase (EC 1.11.1.6), catecholase (catechol oxidase: EC 1.10.3.1) and phenoxazinone synthase (o-aminophenol oxidase: EC 1.10.3.4) are the members of his group. Catecholase and phenoxazinone synthase contain Cu(II), while catalase contain Fe(II) in their active sites.

In this study, we investigated previously synthesized and characterized[19] homodinuclear (**1**) Co(II), (**2**) Ni(II), (**3**) Cu(II), (**4**) Zn(II) metal complexes' three different enzymatic activities namely; catalase, catecholase and phenoxazinone synthase. Enzymes are picked up due to their biochemical importance and the mimicking capacity of our complexes. Catalase is responsible for disproportionation reaction of potentially harmful hydrogen peroxide to oxygen and water.[20] Likewise, catecholase enzyme catalyzes the conversion of 3,5-di-*tert*-butylcatechol to 3,5-di-*tert*-butylquinone[21]. Finally phenoxazinone synthase catalyzes the formation of 2-aminophenoxazine-3-one (APX) from 2-aminophenol (OAPH).[22] All three enzymes are metabolically important as well as the substrates and the products.

2. MATERIALS AND METHODS

2.1. Physical Measurements

All the reagents and solvents were of reagent-grade quality and purchased from commercial suppliers. The UV-Vis measurements were done on a PG T80+ spectrophotometer.

2.2. Catalase-like Activity

Volumetric measurements of evolved dioxygen during the disproportionation reactions of the H_2O_2 with homodinuclear complexes were modified and applied from Kaizer *et al*. as follows: A 50 cm^3 three-necked round-bottom flask containing a solution of the complexes (0.005 mmol solid sample) in DMF (10 cm^3) 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 being stirred, H_2O_2 (1.33 mmol, 0.150 cm^3) was injected into it through the rubber septum using a microsyringe. Volumes of evolved dioxygen were measured at 1 min intervals by volumetry[17] Pyridine (50 mg) was introduced into the reaction vessel as an accelerator, before the addition of H_2O_2. Observed initial rates were expressed as $moldm^{-3}s^{-1}$ by taking the volume of the solution (10 cm^3) into account and calculated from the maximum slope of the curve describing the evolution of O_2 versus time.

2.3. Catecholase-like Activity

The catalytic oxidation of the model substrate 3,5-DTBC (3,5-di-*tert*-butylcatechol) was evaluated by homodinuclear Co(II), Ni(II), Cu(II) and Zn(II) complexes spectrophotometrically in dioxygen-saturated methanol by monitoring the increase in absorbance at 400 nm, corresponding to the formation of the quinone product 3,5-DTBQ (3,5-di-*tert*-butyl-o-benzoquinone).[23] The observed rate constant (k_{obs}) values of the metal complexes for the 3,5-DTBQ formation were obtained from eq (1).

$$\ln(A_\infty/A_\infty\text{-}A_t)=(k_{obs})_1 t \qquad \text{eq (1)}$$

A_∞ and A_t are the absorbance of the formed 3,5-DTBQ at time = ∞ and time = t, respectively.

2.4. Phenoxazinone Synthase-like Activity

The reaction between 2-aminophenol (OAPH) and dioxygen in the presence of catalytic amount of homodinuclear Co(II), Ni(II), Cu(II) and Zn(II) complexes was performed. Complexes (1.67 x 10-4 M each) and correspondingly the substrate; 2-aminophenol (OAPH) (12.5 x 10-3 M) were dissolved and completed to 25 mL of DMF as the final volume. Spectrum scan was carried out between 300-600 nm with 30 sec intervals for 25 repeats. The oxidation reaction of OAPH was monitored by following the increase in absorbance at 433 nm by UV–Vis spectrophotometer, which is a typical band for 2-aminophenoxazine-3-one (APX).[22]

The observed rate constant (k_{obs}) values of the metal complexes for the

e-mail: bulentdede@sdu.edu.tr

APX formation were obtained from eq (2).

$$\ln(A_\infty/A_\infty - A_t) = (k_{obs})_2 t \qquad \text{eq (2)}$$

A_∞ and A_t are the absorbance of the formed APX at time = ∞ and time = t, respectively.

3. RESULTS AND DISCUSSION

Complexes, investigated for their catalytic activity, were synthesized as described before.[19] They were also verified for their physical and spectroscopic properties which explained before by our research group. All complexes were formed in the mole ratio of 2:2 metal:ligand (Figure 1). Results obtained were compatible with the previous data.[19]

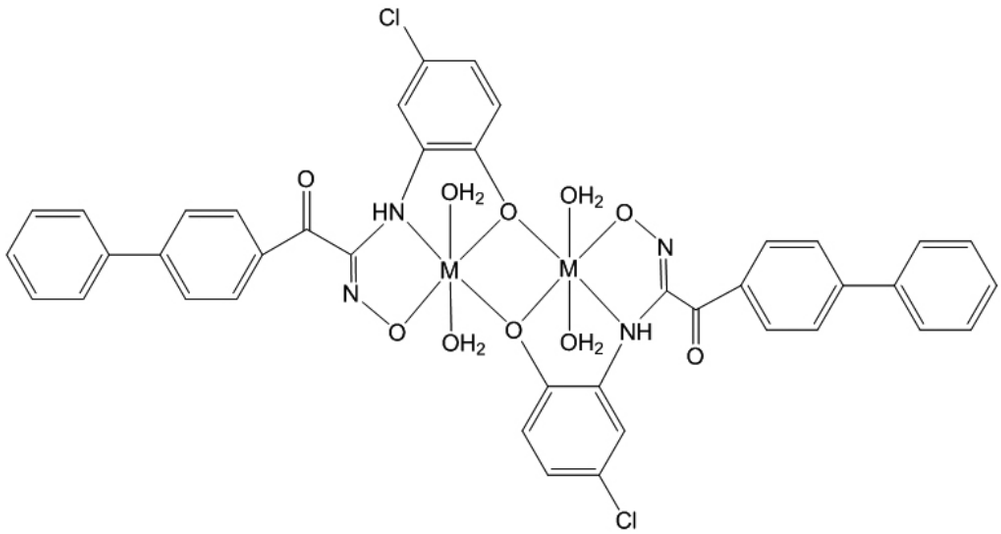


Figure 1. General structure of homodinuclear metal complexes.

[M:(**1**) Co(II), (**2**) Ni(II), (**3**) Cu(II), (**4**) Zn(II)]

3.1. Catalase-like Activity

These complexes were tested for their mimicking capacity of catalytic activity of catalase, catecholase and phenoxazinone synthase enzymes.

Homodinuclear Cu(II) and Mn(II) complexes are used rather than the heterodinuclear ones for the catalase-like activity studies by researchers. Synthesized homodinuclear Co(II), Ni(II), Cu(II) and Zn(II) complexes have been studied for catalytic activity in decomposing H_2O_2 at 25ºC by volumetric measurements. The catalytic activity of the complex towards hydrogen peroxide was investigated in N,N-dimethylformamide (DMF). In the absence of pyridine as a heterocyclic base, the complex disproportionated hydrogen peroxide slowly. The time course of the O_2 evolution can be seen from Figure 2.

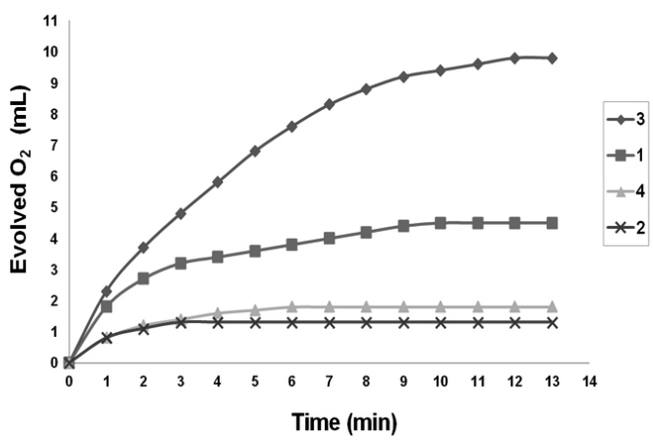


Figure 2. Increase in O_2 yield resulted from H_2O_2 disproportion

Catalase mimicking studies have been repeated several times to ensure consistency of the results.

$$2H_2O_2 \xrightarrow{\text{catalyst}} 2H_2O + O_2$$

The disproportion reaction of peroxide is completed up to 13 minutes. For one molecule of complexes, corresponding number of H_2O_2 disproportionated has been found 28, 13, 36, 13 for Co(II), Ni(II), Cu(II) and Zn(II) at the 1st minute of the reaction and Co(II), Ni(II), Cu(II) and Zn(II) reached the highest number of disproportionated H_2O_2 as 71, 20, 154, 28 at 10th, 3rd, 13th and 6th minutes, respectively.

For all complexes, the reaction rate of the first minute is faster than the succeeding minutes. The rapid dioxygen evolution rate reached as soon as the reaction was initiated and slowed down as the reaction proceeded which can be attributed partially to occupation of the complex molecules. Initial rates of the reactions have been calculated and showed in Table 1. Cu(II) homodimer complex showed the highest catalytic activity among all complexes. The catalytic efficiency of the complexes at first minute was obtained as 3>1>4=2.

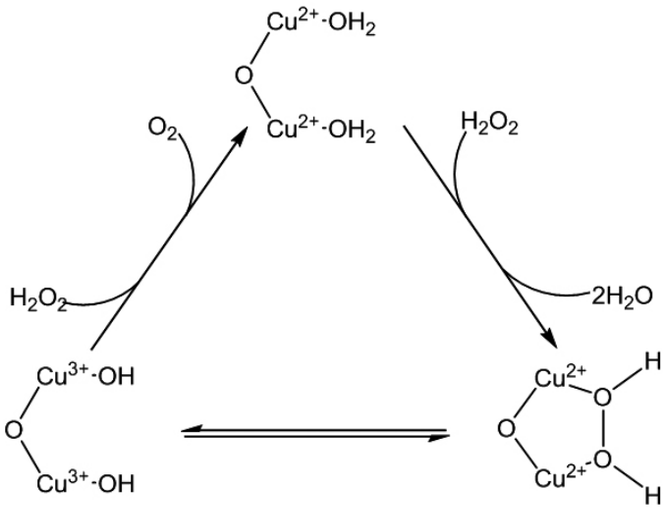
Table 1. Initial reaction rates of complexes 1-4 for the disproportion of H_2O_2

Complexes	Rate ($moldm^{-3}s^{-1}$)
(1) Co(II)	1.18×10^{-5}
(2) Ni(II)	0.53×10^{-5}
(3) Cu(II)	1.51×10^{-5}
(4) Zn(II)	0.53×10^{-5}

Catalase-like activity results of our complexes are comparable with the previous results. Gao *et al*. studied 3 different dinuclear Cu(II) complexes and expressed lower rate values ranging from $1.35x10^{-5}$ to $0.2x10^{-5}$ in $moldm^{-3}s^{-1}$.[24] Similarly, Pires *et al*. studied mononuclear Co(III), Cu(II) and Ni(II) complexes and obtained the best activity from Ni(II) complex. They found Ni(II) complex's initial rate value as ($2.0x10^{-7}$ $moldm^{-3}s^{-1}$) which was 1.7 times higher than their previous study containing tetranuclear Fe(III) complex.[25] When compared to previous studies performed in similar experimental conditions and expressed in similar units, our complexes showed higher and, regarding some studies, significantly higher catalase-like activity. On the other hand, our complexes have far more lower catalytic efficiency compared to natural catalase enzyme.[26]

The proposed reaction mechanism for disproprotionation of hydrogen peroxide can be seen from Scheme 1. At the first step of the reaction, hydrogen

peroxide attacks the complex resulting in the removal of two molecules of coordinated water. In the next step, while Cu(II) is oxidized to Cu(III), O-O bond of hydrogen peroxide goes through a homolytic breakdown forming hydroxyl radical-Cu(III) moieties. Cu(III)-OH intermediate goes back to its original conformation by taking another hydrogen peroxide yielding a molecule of oxygen and coordination waters bonded back to Cu(II) atom.



Scheme 1. A proposed mechanism for catalase-like enzyme activity of the complexes

3.2. Catecholase-like Activity

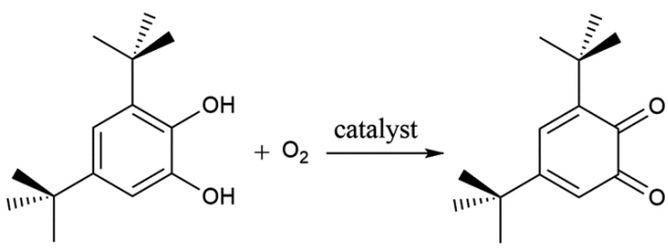


Figure 3. Oxidation reaction of the 3,5-di-tert-butylcatechol by catecholase enzyme to the 3,5-di-tert-butylquinone in the presence of O_2

All complexes were also tested for their catecholase-like activity spectrophotometrically by following the increase in absorbance at 400 nm which is characteristic for 3,5-di-*tert*-butylquinone. Overall reaction can be seen in Figure 3. Methanol is used as a solvent due to good solubility of the substrate and product as well as the complexes. In Figure 4, dinuclear Cu(II) complex spectrum scan was chosen to exhibit since it exerted the highest activity among four synthesized complexes. A gradual increase in absorbance value at 400 nm has been shown by the measurements taken 30 sec. intervals. Other complexes showed the same pattern of spectrum scan with lower absorbance values.

Figure 5 shows the relative observed rate constants (k_{obs}) of the complexes which are equal to the slope of the best lines. According to the results, homodinuclear Cu(II) complex exerted the highest activity revealed by the highest slope (k_{obs}=0.143 min^{-1}). The relative rates of the complexes may be shown like 3>1>2>4 which correspond to; Cu(II), Co(II), Ni(II) and Zn(II), respectively.

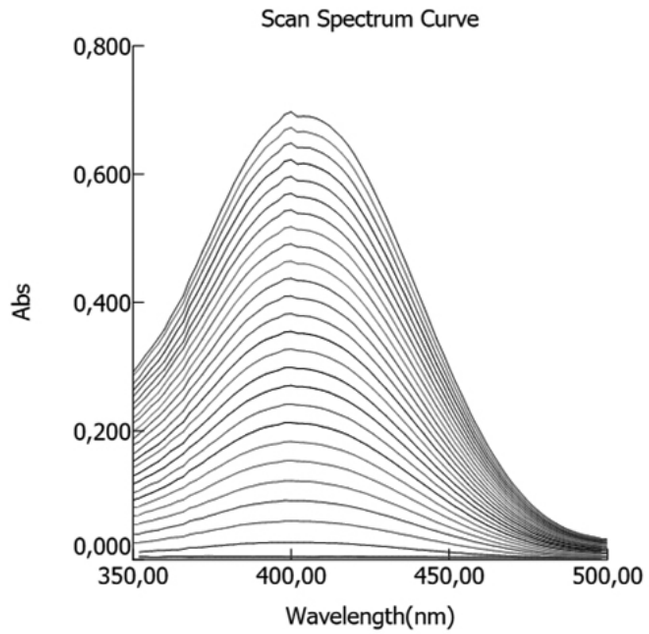


Figure 4. Spectrum scan of catecholase catalyzed reaction taken by 30 sec. intervals. The increase in absorbance at 400 nm is characteristic for 3,5-di-*tert*-butylquinone

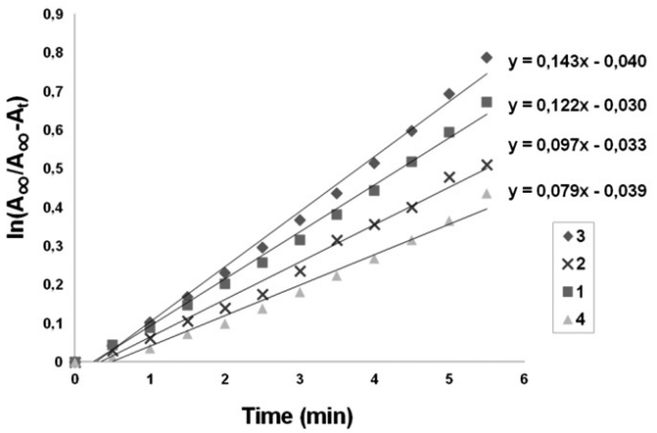


Figure 5. Comparison of relative observed rates of complexes for catecholase-like activity

[(**1**) Co(II), (**2**) Ni(II), (**3**) Cu(II), (**4**) Zn(II)]

Compared to previous studies, our complexes showed higher and lower catecholase-like catalytic efficiency. Chen *et al.* studied mononuclear Cu(II) complex and compared to previously synthesized dinuclear Cu(II) complex's catecholase-like activity. They found k_{obs}(min^{-1}) values ranging from 0.0418 to 0.0545 min^{-1} in different temperature, pH and complex concentration for mononuclear and from 0.0396 to 0.0541 min^{-1} for dinuclear Cu(II) complex.[27] Neves *et al.* studied dinuclear mixed valence Fe(III) and Fe(II) complex and obtained higher k_{obs} values (0.696 min^{-1}) compared to our results (0.143 min^{-1} for Cu(II)).[28]

The reaction mechanism for catecholase described below in three steps starts with the bonding of 3,5-DTBC with dinuclear complex releasing two hydrogens. In this step, Cu(II) ions of the complex are reduced to Cu(I). In the second step which resembles a redox cycle, Cu(I) reacts with O_2 forming the Cu(II)$(O_2)^{2-}$ intermediate. Then, this intermediate reacts with two protons to form a complex with original conformation and a molecule of hydrogen peroxide. This reaction mechanism is experimentally evidenced by Chyn and Urbach.[29]

$$2Cu(II) + 3,5\text{-DTBC} \longrightarrow 2Cu(I) + 3,5\text{-DTBQ} + 2H^{+} \quad (1)$$

$$2Cu(I) + O_2 \longrightarrow 2Cu(II)(O_2)^{2-} \quad (2)$$

$$2Cu(II)(O_2)^{2-} + 2H^{+} \longrightarrow 2Cu(II) + H_2O_2 \quad (3)$$

3.3. Phenoxazinone synthase-like activity

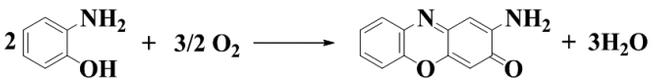


Figure 6. Formation of 2-aminophenoxazine-3-one (APX) from 2-aminophenol (OAPH)

The oxidation of OAPH to APX in the presence of O_2 by complexes (1), (2), (3) and (4) in DMF as a solvent at room temperature was shown in Figure 6. The proportion of OAPH to O_2 was indicated as 2:1,5.

The time evolution of Uv-Vis spectra is shown in Figure 7. Spectral data reveals the time course formation of the product APX. As the other enzyme-like activities dinuclear Cu(II) complex showed the highest activity for phenoxazinone synthase. Generally, a range of wavelength (410-440) is given for APX,[30] we preferred to take the highest absorbance value for rate calculations which is 433 nm. To eliminate the autooxidation of OAPH, we previously tested the same experiment without the complex which gave insignificant increase at 433 nm.

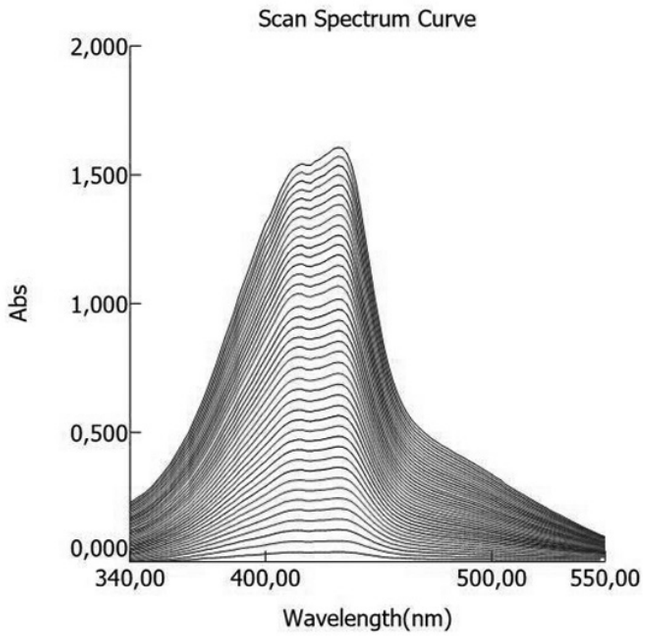


Figure 7. Time evolution UV-Vis spectra of APX formation from OAPH catalyzed by dinuclear Cu(II) complex in the presence of O_2. Spectra were taken 30 sec intervals

The observed rate constant (k_{obs}) values of the metal complexes for the APX formation were obtained from eq (2). Rate constant values obtained from the slope of the best line in Figure 8. The highest activity is shown by complex (3) (k_{obs}=0.214) as in catalase and catecholase-like reactions. Different from catecholase, complex (4) showed insignificantly higher activity compared to complex (2). The catalytic efficiencies of the complexes were as 3>1>4>2.

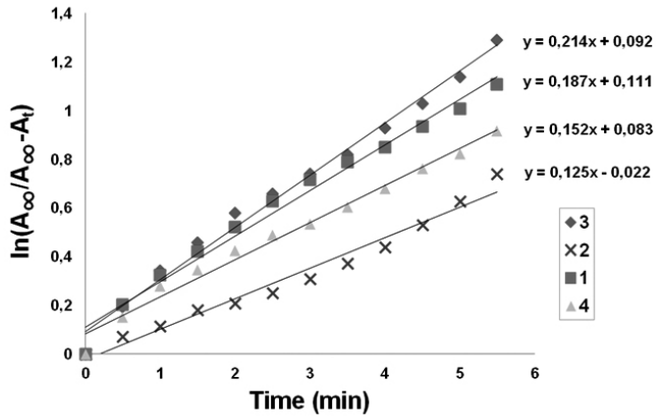


Figure 8. Comparison of relative observed rate of complexes for catalytic oxidation of OAPH to APX

[(**1**) Co(II), (**2**) Ni(II), (**3**) Cu(II), (**4**) Zn(II)]

Complexes (1), (2), (3), (4) showed comparable results. Recently, Mitra *et al.* studied mononuclear Co(III), tetranuclear Cu(II) complex's phenoxazine synthase-like activity. They obtained k_{obs} value as 0.03787 min^{-1} and 0.0188 min^{-1} which are approximately 5 times lower than our Co(II) and 11 times lower than Cu(II) complex, respectively.[31,32]

The mechanism of OAPH oxidation catalyzed by the metal complexes contains steps (1)-(4) and begins with the binding of the OAPH to the metallic center. This intermediate decomposes subsequently and produces OAP• free radical intermediate which is the rate-controlling step (2). 2-aminophenoxy radical was detected[30,33] and unable to detect[34] due to fast disproportionation by the other researchers using ESR spectroscopy. Fast disproportionation of OAP• radical results in the formation of 2-benzoquinone monoimine (BQMI) intermediate. The catalytic reaction sequence is completed by reaction of 2-benzoquinone monoimine with another mole of OAPH which gives the final product APX.[35]

$$OAPH + Cu(II)....Cu(II) \longrightarrow OAPH\text{-}Cu(II)....Cu(II) \quad (1)$$

$$OAPH\text{-}Cu(II)....Cu(II) \longrightarrow {\cdot}OAP\ (radical) \quad (2)$$

$${\cdot}OAP \longrightarrow BQMI \quad (3)$$

$$BQMI + OAPH \xrightarrow{[O_2]} Phenoxazinone\ (APX) \quad (4)$$

4. CONCLUSION

Our study revealed that four different homodinuclear complexes showed relatively high catalytic activities mimicking catalase, catecholase and phenoxazinone synthase compared to the previous studies. Higher activity of the complexes, especially Cu(II), may be due to their dinuclear nature confirming that the dinuclear complexes show higher activity compared to the mononuclear ones of the same metal ions. Other factors for obtained results may be the proximity and right orientation of our complexes with the substrates which are obvious in coherence of catechol and dinuclear complex. Among the studied four, Cu(II) pair containing complex showed the highest activity for three oxidoreductase enzymes. Compared to other metal ions, copper has relatively lower redox potential which may be responsible for the higher activity. Among enzymes that catalyze oxidoreductase reactions, copper is one of the most abundant metal ion acting as a cofactor. Besides, two of the subjected enzymes of this study namely; catecholase and phenoxazinone synthase bear copper ion. Considering all these properties, oxime group containing dinuclear Cu(II) complexes have the potential to be a model for enzyme-like activity studies.

REFERENCES

1. Y. Lu, J. S. Valentine, Curr. Opin. Struct. Biol. 7(4), 495, (1997)
2. S. N. Raja, H. Y. Shrivastava, B. U. Nair, Ind. J. Chem, 50A, 531, (2011)
3. R. K. Sharma, V. Krishnan, Bioinorganic Chemistry, NSDL, India, 2007
4. E. M. Peck, B. D. Smith, Synthetic Receptors for Biomolecules: Design Principles and Applications, RSC Publications, 2015
5. Z. Dong, Q. Luoa, J. Liu, Artificial enzymes based on supramolecular scaffolds, Chem. Soc. Rev.41, 7890, (2012)
6. J. Paschke, M. Kirsch, H.-G. Korth, H. Groot, R. Sustmann, J. Am. Chem. Soc. 123, 11099, (2001)
7. F. Yu, V. M. Cangelosi, M. L. Zastrow, M. Tegoni, J. S. Plegaria, A. G. Tebo, C. S. Mocny, L. Ruckthong, H. Qayyum, V. L. Pecoraro, Protein Design: Toward Functional Metalloenzymes, Chem. Rev. 114, 3495, (2014)
8. B. Dede, F. Karipcin, M. Cengiz, J. Hazard. Mater. 163, 1148, (2009)
9. A. Chakravorty, Coordin. Chem. Rev. 13, 1, (1974)
10. P. Mitchell, Science, 206, 1148, (1979)
11. M. C. M. Laranjeira, R.A. Marusak, A.G. Lappin, Inorg. Chim. Acta. 300, 186, (2000)
12. J. R. Dilworth, S. Parrott, Chem. Soc. Rev. 27, 43, (1998)
13. S. S. Jurisson, J. D. Lydon, Chem. Rev. 99, 2205, (1999)
14. M. Bakir, J. A. M. McKenzie, J. Chem. Soc. Dalton Trans. 3571, (1997)
15. R. Ruiz, F. Lloret, M. Julve, J. Faus, M. C. Munoz, X. Solans, Inorg. Chim. Acta, 213, 268, (1993)
16. N. Sengottuvelan, J. Manonmani, M. Kandaswamy, Polyhedron, 21, 2767, (2002)
17. J. Kaizer, R. Csonka, G. Speier, M. Giorgi, M. Reglier, J. Mol. Catal. A: Chem. 236, 12, (2005)
18. V. Balzani, A. Juris, M. Venturi, S. Campagna, S. Serroni, Chem. Rev. 96, 759, (1996)
19. F. Karipcin, B. Dede, M. Cengiz, Russ. J. Inorg. Chem. 55, 530, (2010)
20. H. Aebi, Method. Enzymol. 105, 121, (1984)
21. M. U. Triller, D. Pursche, W. Y. Hsieh, V. L. Pecoraro, A. Rompel, B. Krebs, Inorg. Chem. 42, 6274, (2003)
22. J. Kaizer, G. Baráth, R. Csonka, G. Speier, L. Korecz, A. Rockenbauer, L. Párkányi, J. Inorg. Biochem. 102, 773, (2008)
23. J, Reim, B. Krebs, J. Chem. Soc. Dalton. Trans. 3793, (1997)
24. J. Gao, A. E. Martell, J. H. Reibenspies, Inorg. Chim. Acta. 346, 32, (2003)
25. B. M. Pires, D. M. Silva, L. C. Visentin, B. L. Rodrigues, N. M. F. Carvalho, R. B. Faria, PLoS. ONE 10, 1, (2015)
26. M. Shank, V. Barynin, G. C. Dismukes, Biochemistry-US, 33, 15433 (1994)
27. Z-F. Chen, Z-R. Liao, D-F. Li, W-K. Li; X-G. Meng J. Inorg. Biochem, 98, 1315, (2004)
28. A. Neves, A. J. Bortoluzzi, R. Jovito, R. A. Peralta, B. de Souza, B. Szpoganicz, A. C. Joussef, H. Terenzi, P. C. Severino, F. L. Fischer, G. Schenk, M. J. Riley, S. J. Smith, L R. Gahan, J. Braz. Chem. Soc. 21, 1201, (2010)
29. J. P. Chyn, F. L. Urbach, Inorg. Chim. Acta. 189, 157, (1991)
30. I. C. Szigyarto, T. M. Simandi, L. I. Simandi, L. Korecz, N. Nagy, J. Mol. Catal. A: Chem. 251, 270, (2006)
31. M. Mitra, R. Ghosh, Indian J. Chem. A. 55, 681, (2016)
32. M. Mitra, T. Kundu, G. Kaur, G. Sharma, A. R. Choudhury, Y. Singhd, R Ghosh, Roy. Soc. Ch. Adv. 6, 58831, (2016)
33. L. I. Simándi, T. M. Barna, L. Korecz, A. Rockenbauer, Tetrahedron Lett. 34, 717, (1993)
34. A. Panja, M. Shyamal, A. Saha, T. K. Mandal, Dalton Trans. 43, 5443, (2014)
35. T. Horváth, J. Kaizer, G. Speier, J. Mol. Catal. A: Chem. 215, 9, (2004)