EFFECT OF pH ON TURNOVER NUMBER OF A BINUCLEAR AND MONONUCLEAR COMPLEX: A COMPARATIVE STUDY

Arnab Chatterjee[1], Samya Neogi[2], Ujjal Kar[3], Bholanath Ghosh[4], Kaushik Mukherjee[5]
Department of Basic Science and Humanities
Dr. B. C. Roy Polytechnic, Durgapur – 713206. West Bengal, India.
Email id – arnab.chatterjee@bcrec.ac.in, samya.neogi@bcrec.ac.in, ujjal.kar@bcrec.ac.in, bholanath.ghosh@bcrec.ac.in, kaushik.mukherjee@bcrec.ac.in

Abstract: ***A reported binuclear Cu(II) complex of the type [$Cu_2(L1)_2$] [L1 = 2- Hydroxy-3-[(2-hydroxy-ethylimino)-methyl]-5-methyl-benzaldehyde] and another reported mononuclear Cu(II) complex [$Cu(L2)_2$](ClO_4)$_2$ [L2 = 2-[(phenyl-pyridine-2-yl-methylene)-amino]-ethanol] were investigated for pH dependent catecholase activity in which 3,5-di-tertiary-butylcatechol was oxidized to 3,5-di-tert-butyl-o-benzoquinone derivative, in methanol (MeOH) solvent. A comparison was made with reference to the turnover numbers for the catalytic activity in basic medium for the mononuclear and the dinuclear complexes.***

Keywords: ***Cu^{II}, Schiff base ligand, catecholase enzyme like activity, pH dependence on catalytic activity***

1. INTRODUCTION

Modeling copper proteins, that can bind or can activate dioxygen for simulating important biochemical reactions, which are oxidizing in nature, is a flourishing area of research [1]. Copper proteins are excellent catalysts in biological systems, crossing the barriers (which are kinetic in nature) related to molecular oxygen's spin functionality. Type 3 copper proteins includes catechol oxidase, tyrosinase and hemocyanin which contains binuclear coppers that are linked magnetically [2]. Tyrosinase catalyses the hydroxylation of phenols followed by their oxidation to quinone derivatives, which then transform to melanin pigment in plants. Catecholase in plants exclusively converts *o*-diphenols to their respective quinones only. A schematic representation of the catecholase activity is presented in figure 1. Hemocyanin is responsible for oxygen transport and its consequent storage in some invertibrates such as arthopods and mollusks. Soon after the crystal structures of tyrosinase [3] and catecholase [4] were published in the literature, scientists all over the world began modelling the enzymes present in nature [5]. Though catecholase contains a dicopper active site, a number of monocopper [6] and non-copper [7] complexes with similar activity have been discovered. In the present work, we have prepared a reported binuclear Cu (II) [8] and a reported mononuclear Cu (II) [9] complex that showed catecholase activity in methanolic medium and investigated the effect of pH on the substrates. A comparision was tried to be shown on the pronounced effect pH on dinuclear and mononuclear complexes.

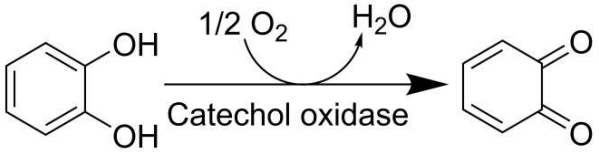

Figure 1: ***Catecholase activity in presence of aerial oxygen***

2. EXPERIMENTAL PROCEDURE

2.1. Methods and materials

The reagents were obtained from various chemical suppliers and used exactly as they were given. Before usage, solvents were distilled after being dried according to conventional practices. 2,6-diformylparacresol, used as a starting material for the preparation of L1, was prepared according to a reported procedure [10]. For the study of catecholase like activity, 10^{-4} mol dm^{-3} of a basic solution of **1** was treated with 10^{-2} mol dm^{-3} of 3,5-di-tertiarybutylcatechol under aerobic conditions. L2 was prepared by the classical condensation reaction of 2-benzoylpyridine and 2- aminoethanol. The catecholase activity of **2** was investigated in two separate ways; using a solution of **2** in MeOH and another in basic condition in MeOH.

*2.2 Synthesis of **1***

As described in published literature, 2,6-diformylparacresol was prepared [10]. The ligand (L1) was prepared by the reaction among 2,6-diformylparacresol (0.05002 g, 0.30 millimole) with 2-aminoethanol (0.03702 mg, 0.60 millimole) in ethanolic (EtOH) medium. **1** was prepared by *in situ* addition of Cu $(NO_3)_2.6H_2O$ (0.09407 g, 0.5 millimole) to L1 (Figure 2)

Yield (Based on amount of metal salt taken): 0.174 g (51%), Important IR bands (Fig. S1, ESI) (By KBr pellet method, cm^{-1}): 1561, 1631, 3101–3401; UV–Vis (Fig. S2, ESI) (λ nanometre, MeOH): 217, 251, 391, 661; Carbon Hydrogen Nitrogen (CHN) Analysis: Calculated: $C_{22}H_{28}N_4O_{14}Cu_2$: C, 37.770%; H, 4.031%; N, 8.012%. Found, C, 37.722%; H, 3.991%; N, 8.122%.

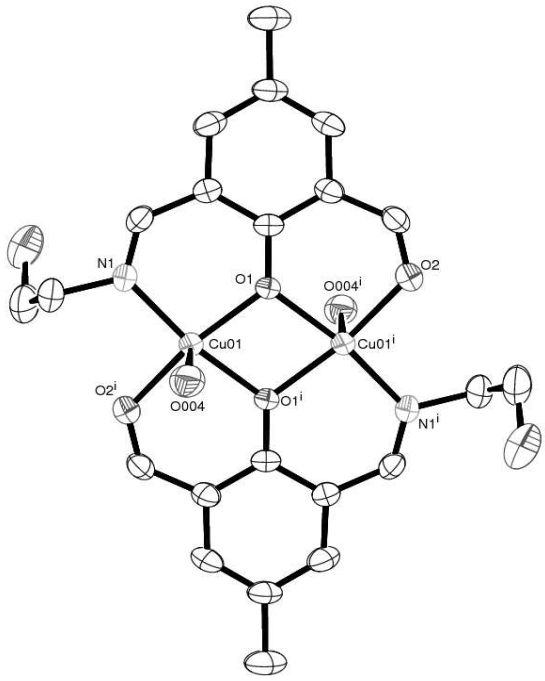

Figure 2: ***ORTEP of 1 (40% ellipsoid probability for non-hydrogen atoms)***

*2.3 Synthesis of L2 and **2***

In a 250 millilitre rb flask, 5.451 millimole (0.100 g) of phenyl(pyridin-2-yl)methanone (commonly known as 2-benzoylpyridine) in almost 100 mL of EtOH was taken followed by addition of 5.452 millimole (0.3221 g) of ethanol amine (also known as 2-aminoethanol). Refluxing the ensuing reaction mixture for seven hours produced the ligand L2. Characterization of the product (L2) was done using various spectroscopic techniques like NMR, IR and UV.

Yield (based on the amount of 2-benzoylpyridine taken): 0.8918 g (72.30%). ^{1}HNMR (Fig. S3, ESI) ($CDCl_3$, 500 MHz, δ_H, ppm) 2.55 (t, 1H, J = 7.92 Hz), 3.77 (t, 1H, J = 6.25 Hz), 3.73 (t, 1H, J = 6.25 Hz), 3.73 (t, 2H, J = 6.25 Hz), 6.94–8.53 (m, 9H). Important IR bands (KBr pellet method, cm^{-1}) (Fig. S4; ESI): 1671, 3351; UV–Vis (λ nanometer, MeOH) (Fig. S5; ESI): 354, 245.

0.451 millimole (0.1672 g) of Cu $(ClO_4)_2.6H_2O$ was added to 20 millilitre of MeOH in a 50 mL beaker, to which 0.451 millimole (0.1001 g) of the ligand. The solution was stirred for about 60 minutes. Upon filtering and keeping the resultant solution for crystallization, greenish blue crystals were obtained. The crystals were collected and dried in air. ORTEP of 2 is given in Figure 3.

Yield (based on metal salt): 91 mg (45.00%), Selected IR bands (KBr pellet, cm^{-1}) (Fig.S6; ESI): 1632, 1100, 620, 3300; UV–Vis (λ nm, MeOH) (Fig. S7; ESI): 205, 235, 270, 650.

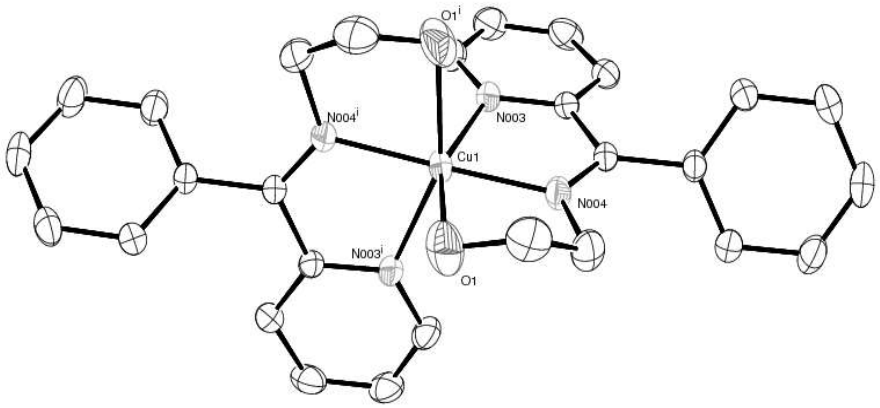

Figure 3: ***ORTEP of 2 with 30% ellipsoid probability for non-hydrogen atoms***

2.3.Physical measurements

IR prestige (Shimadzu) was used to record the IR spectrum. These were recorded using KBr disks in the wavelength of 4000-500 cm^{-1}. UV-Vis spectra were recorded in Shimadzu UV-Vis 2450 spectrophotometer in appropriate solvents. Nuclear magnetic resonance data (^{1}H) were collected on Bruker instrument (400 MHz) using deuterated chloroform ($CDCl_3$) as dissolution medium.

3. RESULTS AND DISCUSSIONS

3.1 Synthesis and formulation

Refluxing of 2,6-diformylparacresol with 2-aminoethanol in EtOH for 5h resulted in the formation of L1. The resulting solution was used to react with Cu $(NO_3)_2.6H_2O$ for the *in situ* generation of **1**. The IR spectrum of 1 at around 1631 cm^{-1} indicated the formation of the metal complex. (Figure S1, ESI).

L2 was synthesized by the reaction between 2-aminoethanol and 2-benzoylpyridine in EtOH for 7h. The resulting reaction mixture was evaporated in a water bath to obtain the product. Characterizations of product were done by various methods like IR, UV. The IR stretching band at around 1671 cm^{-1} indicated the formation of the imine bond (Figure S4, ESI). **2** was obtained by refluxing L2 with Cu $(ClO_4)_2.6H_2O$ in methanol. Crystalline compounds were generated and characterized. The IR spectrum at around 1632 cm^{-1} indicated complex formation of the ligand with copper (Figure S6, ESI). The UV-Vis spectrum for **2** was recorded in MeOH and the absorption peaks at 205 nm, 235 nm, 270 nm and 650 nm were obtained.

3.2 X-ray structure

The IR characterization along with other physical characteristics of the synthesized complexes matched exactly with that reported previously by the corresponding author. The identities of the synthesized complexes were further confirmed by mounting the complexes on the goniometer head of Bruker Smart Apex 2, used in structural elucidation. The initially evaluated crystal parameters of both the synthesized complexes came identical to that of the previous reports. The crystallographic table of the previous complexes were reported by Chatterjee *et.al* [8-9] and given in this article.

3.3 Catecholase like activity

3,5-ditertiarybutyl catechol, having two stearically demanding t-butyl groups, was chosen for catecholase activity study as it has lower quinone-catechol reduction potential. The catalyzed product o-benzoquinone derivative shows UV-Vis absorption peak at around 401 nanometer in MeOH [11]. **1** showed absorption bands in the UV-Vis region that came at 217, 251, 391 and 661 nanometre (nm) (Figure S3, ESI) and for **2** the electronic bands came at 205, 235, 270 and 650 nm (Figure S4, ESI), while the absorption band for 3,5-ditertiarybutylcatechol appeared at 282 nm. Our aim to investigate the effect of pH on the activity of reported dinuclear and mononuclear copper complexes was addressed through the tuning of pH of the solutions of **1** and **2** to the basic range as it was reported in literature that acceleration in this enzyme activity was noticed in basic range [12]. For maintaining a pH of around 8.5 for both the complex solutions, we used a mild organic base triethyl amine. The catecholase activity of **1** (in absence of basic medium) was already reported in methanol solvent [8]. The catecholase activity of **2** was assessed in MeOH. Reaction of 100 equivalents of 3,5-ditertiarybutylcatechol with basic solutions of **1** and **2** separately in MeOH, the UV-Vis spectrum was obtained by repetitive mode. Conversion of colourless to deep brown solution after some time interval indicated the transformation of 3,5-ditertiarybutylcatechol to 3,5-ditertiarybutylquinone which was supported by increase in intensity of the band due to formation of quinone at ~ 402 nm for basic solution of **1** and 395 nm for basic solution and methanolic solution of **2** (Figure 4-6). Reaction with increased quantities of the starting materials led to the production of desired 3,5-ditertiarybutylquinone, the same was separated from the unreacted reaction mixture column chromatographically using a mixed solvent (ethyl acetate and hexane in the ratio 1:9) as eluent.

Comparing melting points of the isolated product with that of o-quinone, its identity was confirmed [13]. The assessment of catecholase activity of **2** was carried out in MeOH solvent so that we can compare the effect of pH on the catecholase activity. The various kinetic parameter of the two compounds are discussed in Table 1.

Crystal Data and Structure Refinement Parameters[8,9]		
	1	**2**
Formula	$C_{22}H_{28}N_4O_{14}Cu_2$	C_{28} H_{28} Cl_2 Cu N_4 O_{10}
Mol. Wt.	699.56	714.98
Crystal System	monoclinic	Monoclinic
Space Group	C 2/ c	C 1 2/c 1
Temperature (K)	296(2)	296
Wavelength	0.71073	0.71073
a/Å	16.2073(5)	16.4216(5)
b/Å	12.1215(4)	10.9691(3)
c/Å	14.9607(5)	17.0282(5)
α/°	90	90
β/°	109.510(2)	98.384(1)
γ/°	90	90
V/Å^3	2770.38(16)	3034.51(15)
Z	4	4
D_c/g cm^{-3}	1.677	1.565
μ/mm^{-1}	1.612	0.959
F(000)	1432	1468.0
R(int)	0.0598	0.018
Total reflections	24515	27561
unique reflections	3453	3798
Completeness to theta	0. 992	0.995
Absorption correction	Multi-Scan	multi-scan
T_{max} and T_{min}	0.890 and 0.938	28.41° to 2.42^0
Data/restraints/parameters	3453/ 0 / 201	3798 / 0 / 209
Goodness-of-fit (GOF) on F^2	1.004	1.046
Final R indices [l > 2s(l)]	R_1 =0.0516, wR_2 = 0.1366	R1 = 0.0456, wR2 = 0.1393
R indices (all data)	R_1 =0.0877, wR_2 = 0.1587	R1 = 0.0515, wR2 = 0.1464
Largest difference in peak and hole (e _A_3)	0.791, -0.631	0.587 and -0.461

Table 1: Comparison of kinetic parameter of a basic solution of 1 and 2

Compound	Solvent	K_{cat} (h^{-1})	Std. error
1 (Basic solution)	Methanol	65.49	4.05E-01
2 (Basic solution)	Methanol	30.0	0.70E-01

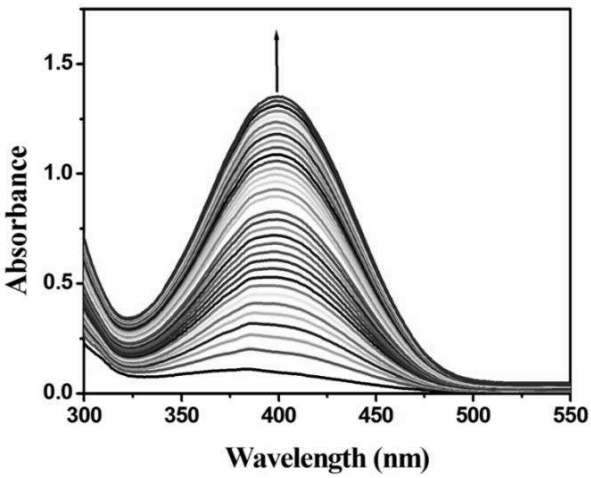

Figure 4: ***Catecholase activity of 1 in MeOH***

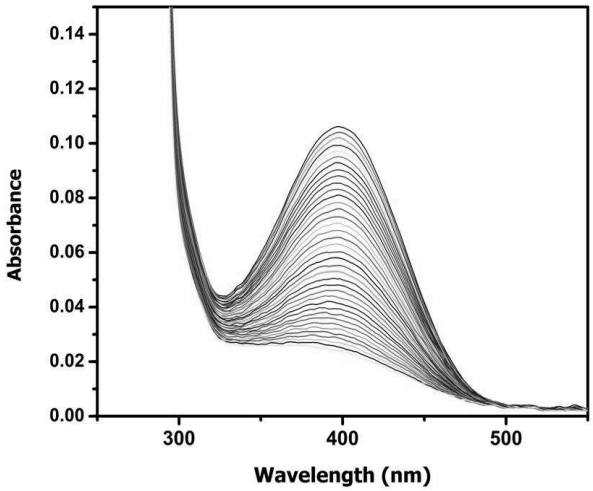

Figure 5: ***Catecholase activity of 2 in MeOH***

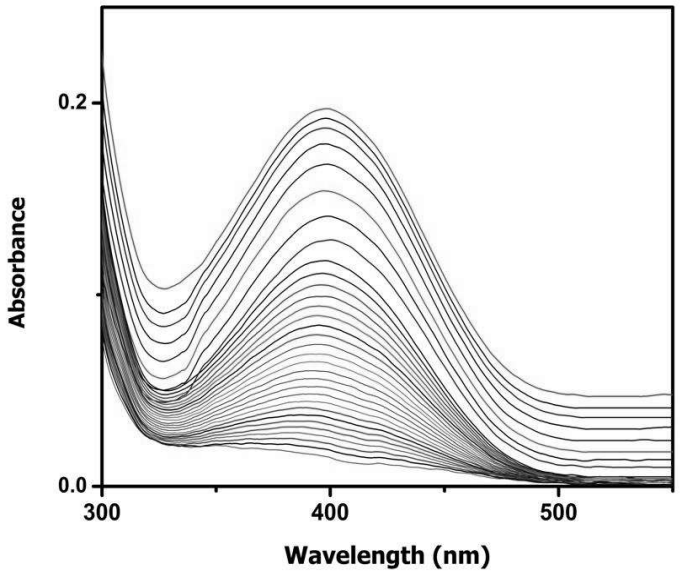

Figure 6: ***Catecholase activity of basic solution of 2 in MeOH***

3.4. Kinetic studies of catecholase like activity

A basic solution of **1** was put through experiments for kinetic studies spectrophotometrically using MeOH with 3,5-ditertiarybutylcatechol. The reaction setup was maintained at a constant temperature of 298 K. A volume of 0.04 ml having a strength of 10^{-4} (M) of the basic solution of **1** was made to react with a volume of 2 ml of 3,5-ditertiarybutylcatechol of a particular concentration (10^{-3} (M) to 10^{-2} (M)), so that the final concentration becomes 1×10^{-4} (M). Transformation of 3,5-ditertiarybutylcatechol to 3,5-ditertiarybutylquinone was analyzed in an UV-Vis spectrophotometer at a wavelength of ~401 nm in MeOH solvent.

Michaelis-Menten method was used to evaluate the important parameters that are related to this kinetic experiment (Figure 7) like turnover number (K_{cat}) which came to be 65.49 h^{-1} in MeOH.

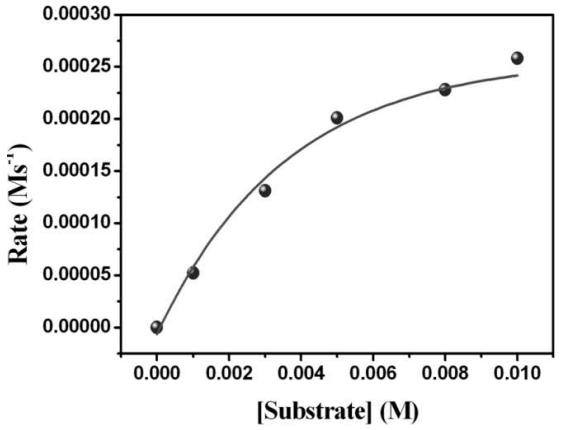

Figure 7. ***Michealis-Menten Kinetics for solution of 1 in MeOH***

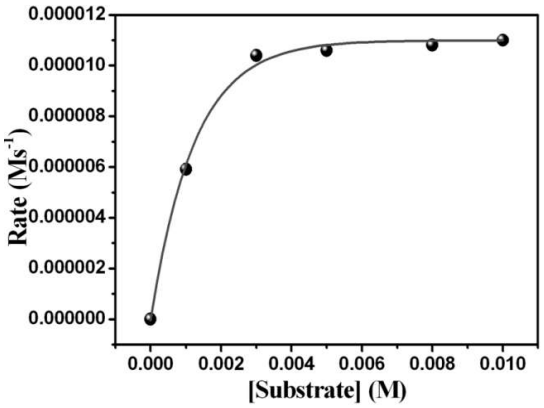

Figure 8. ***Michealis-Menten Kinetics for solution of 2 in MeOH***

The basic solution of **2** was similarly treated with 3,5-ditertiarybutylcatechol in methanol. The reaction setup was thermostated at 298 K. A volume of 0.04 ml having a strength of 10^{-4} (M) of the basic solution of **1** was made to react with a volume of 2 ml of 3,5-ditertiarybytylcatechol of a particular concentration (10^{-3} (M) to 10^{-2} (M)), so that the final concentration becomes 1×10^{-4} (M). Transformation of 3,5-ditertiarybutylcatechol to 3,5-ditertiarybutylquinone was analyzed in an UV-Vis spectrophotometer at a wavelength of ~401 nm in MeOH solvent.

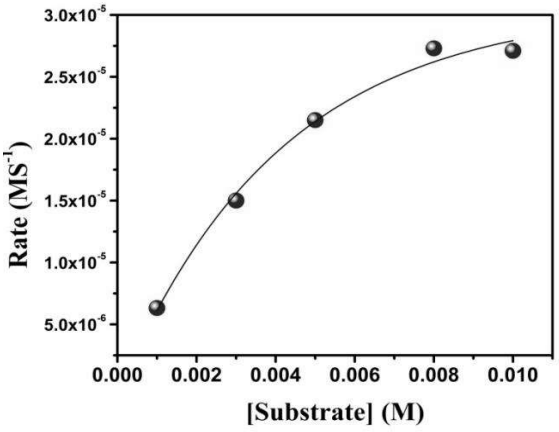

Figure 9. ***Michealis-Menten Kinetics for a basic solution of 2 in MeOH***

Michaelis-Menten method was used to evaluate the important parameters that are related to this kinetic experiment (Figure 8) like turnover number (K_{cat}) which came to be 30.0 h^{-1} in MeOH.

A similar approach to find the various kinetic parameters was carried out using a methanolic solution of **2** (in absence of basic medium) for comparison purpose with the base treated situation (Figure 9). The turnover number of the basic solution of **2** in MeOH was found to be 41.7 h^{-1}.

4. CONCLUSIONS

Synthesis and pH dependent catalytic activity of a dinuclear and a mononuclear Cu (II) complex were done and some interesting observations were noted. The catalytic activity of dinuclear copper complex is always higher compared to the mononuclear analogues. Again, the catecholase activity increased in basic solvents. This is probably because reactions in which oxidation happens is enhanced in a medium that can trap the residual protons. This study can therefore help in designing excellent oxidizing catalysts with high turnover numbers.

REFERENCES

1. (a) Z. Wang, W. Fang, W. Peng, W. Peng and B. Wang, "Recent Computational Insights into the Oxygen Activation by Copper-Dependent Metalloenzymes" Top Catal vol. 65, 2022, pp 187-195; (b) C. E. Elwell, N. L. Gagnon, B. D. Neisen, D. Dhar, A. D. Spaeth, G. M. Yee and W. B. Tolman, "Copper–Oxygen Complexes Revisited: Structures, Spectroscopy, and Reactivity", Chem. Rev. vol. 117, 2017, pp 2059-2017, references therein. (c) W. Peng, X. Qu, S. Shaik and B. Wang, "Deciphering the oxygen activation mechanism at the Cu_C site of particulate methane monooxygenase" Nat. Catal vol. 4, 2021, pp 266-273.

2. (a) Á. Dancsa, N. May, K. Selmeczi, Z. Darula, A. Szorcsik, F. Matyuska, T. Páli and T. Gajdaa, "Tuning the coordination properties of multi-histidine peptides by using a tripodal scaffold: solution chemical study and catechol oxidase mimicking", New J. Chem. Vol. 41, 2021, pp 808-823; (b) P. Zerón, M. Westphal, P. Comba, M. Flores-Álamo, A. C. Stueckl, C. Leal-Cervantes ,V. M. Ugalde-Saldívar and L. Gasque, "Dinuclear Copper(II) Complexes with Distant Metal Centers: Weaker Donor Groups Increase Catecholase Activity", Eur. J. Inorg. Chem. Vol. 56, 2017, pp 56-62.

3. (a) Y. Matoba, T. Kumagai, A. Yamamoto, H. Yoshitsu and M. Sugiyama, "Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis", J. Biol. Chem. Vol. 281, 2006, 8981-8190; (b) H. Decker, T. Schweikardt and F. Tuczek, "The first crystal structure of tyrosinase: all questions answered?", Angew. Chem. Int. Ed. Vol. 45, 2006, pp 4546-4550.

4. T. Klabunde, C. Eicken, J. C. Sacchettini and B. Krebs, "Crystal structure of a plant catechol oxidase containing a dicopper center" Nat. Struct. Biol. Vol. 5, 1998, pp 1084-1090.

5. S. Mandal, J. Mukherjee, F. Lloret and R. Mukherjee, "Modeling Tyrosinase and Catecholase Activity Using New *m*-Xylyl-Based Ligands with Bidentate Alkylamine Terminal Coordination" Inorg. Chem. Vol. 51, 2012, 13148-13161.

6. M. Mitra, A. K. Maji, B. K. Ghosh, G. Kaur, A. R. Choudhury, C.-H. Lin, J. Ribas and R. Ghosh, "Synthesis, crystallographic characterization and catecholase activity of a monocopper(II) and dimanganese(III) complex with an anionic Schiff base ligand", Polyhedron vol. 61, 2013, pp 15-19.

7. M. Das, R. Nasani, M. Saha, S. M. Mobin and S. Mukhopadhyay, "Targeted Water Soluble Copper-tetrazolate Complexes: Interactions with Biomolecules and Catecholase like Activities" Dalton Trans. Vol. 44, 2015, pp 2299.

8. A. Chatterjee, Md. M. Seikh, S. Chowdhury and R. Ghosh, "Catecholase and catechol cleavage activities of a dinuclear phenoxobridged Cu (II) complex: Synthesis, structure and magnetostructural studies", Inorganica Chim. Acta vol. 521, 2021, p 120345.

9. A. Chatterjee, S. Khan and R. Ghosh, "Structurally Characterized Mononuclear Isostructural Ni(II), Cu(II) and Zn(II) Complexes as a Functional Model for Phenoxazinone Synthase Activity" Polyhedron vol. 173, 2019, p 114151.

10. D. A. Denton and H. Suschitzky, "906. Synthetic uses of polyphosphoric acid", J. Chem. Soc., 1963, 4741-4743.

11. K. S. Banu, T. Chattopadhyay, A. Banerjee, S. Bhattacharya, E. Suresh, M. Nethaji, E. Zangrando and D.Das, "Catechol oxidase activity of a series of new dinuclear copper(II) complexes with 3,5-DTBC and TCC as substrates: Syntheses, X-ray crystal structures, spectroscopic characterization of the adducts and kinetic studies" Inorg. Chem, vol. 47, 2008, pp 7083-7093.

12. A. Chatterjee, G. Kaur, M. Joshi, A.R. Choudhury and R. Ghosh, "pH dependent catecholase activity of Fe(II) complexes of type [Fe(L)]X_2 [L = N-(phenyl-pyridin-2-yl-methylene)-ethane-1,2-diamine; X = $ClO4^-$(1), $PF6^-$(2)]: Role of counter anion on turnover number" Inorg. Chim. Acta, vol. 513, 2020, pp 119933.

13. M. Mitra, P. Ragahvaiah and R. Ghosh, "A mononuclear cobalt (III) complex and its catecholase activity", *New J. Chem.*, vol. 39, 2015, pp 200-205.

Effect of pH on turnover number of a dinuclear copper (II) complex (1) $[Cu_2(L1)_2]$ [L1 = 2- Hydroxy-3-[(2-hydroxy-ethylimino)-methyl]-5-methyl-benzaldehyde] and a mononuclear copper (II) complex(2)$[Cu(L2)_2](ClO_4)_2$ [L2 = 2-[(phenyl-pyridine-2-yl-methylene)-amino]-ethanol] with Schiff base ligand: A comparative study

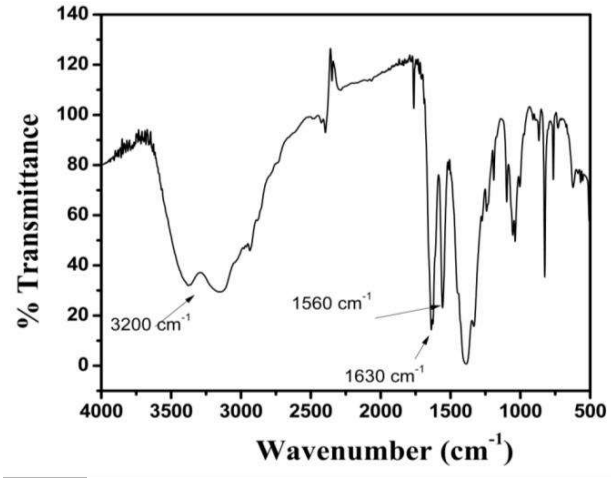

Figure S1: *IR spectrum of 1*

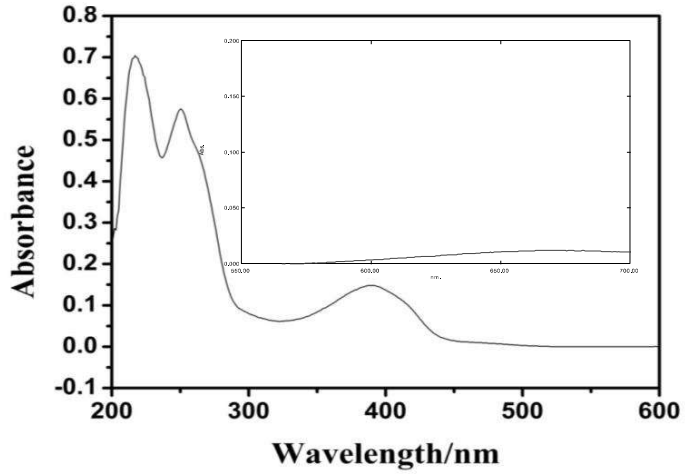

Figure S2: *UV spectrum of 1 in MeOH*

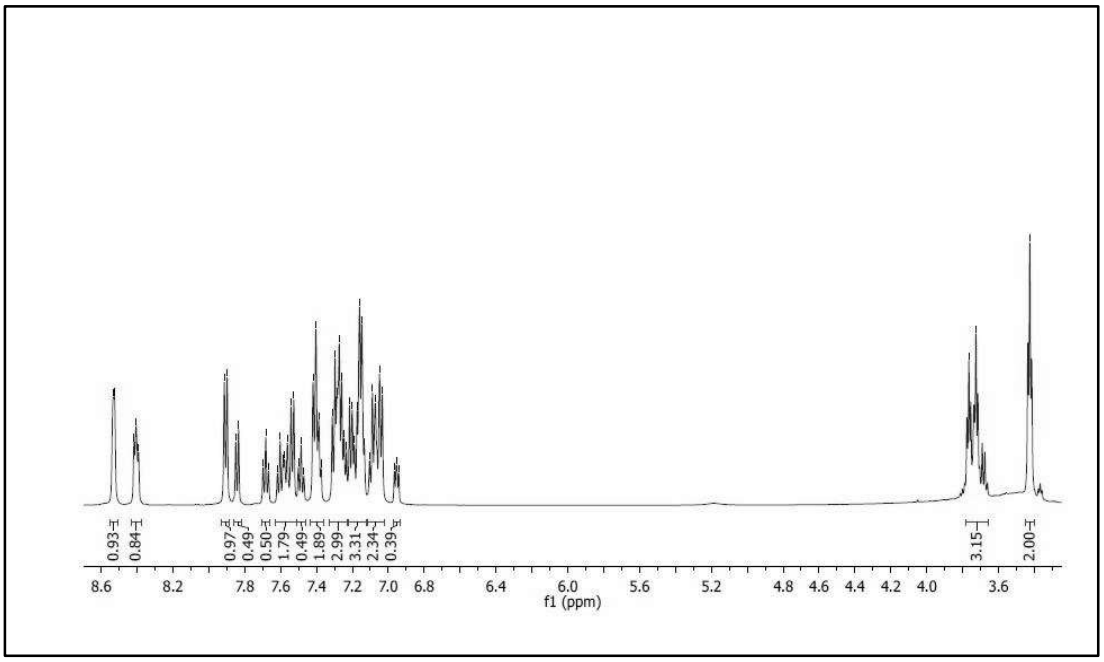

Figure S3: *^{1}H NMR of L2 in $CDCl_3$*

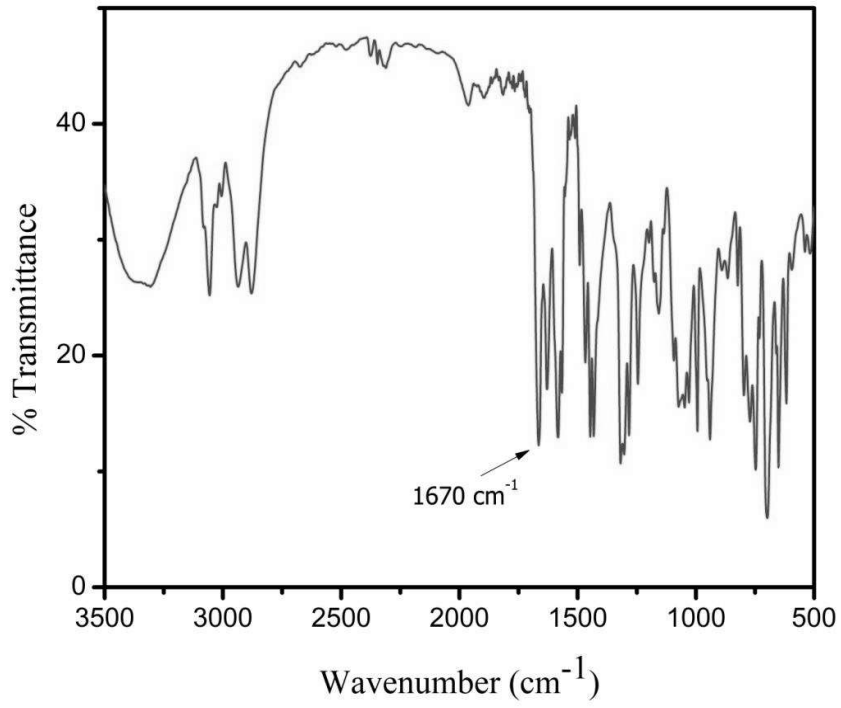

Figure S4: *IR spectrum of L2*

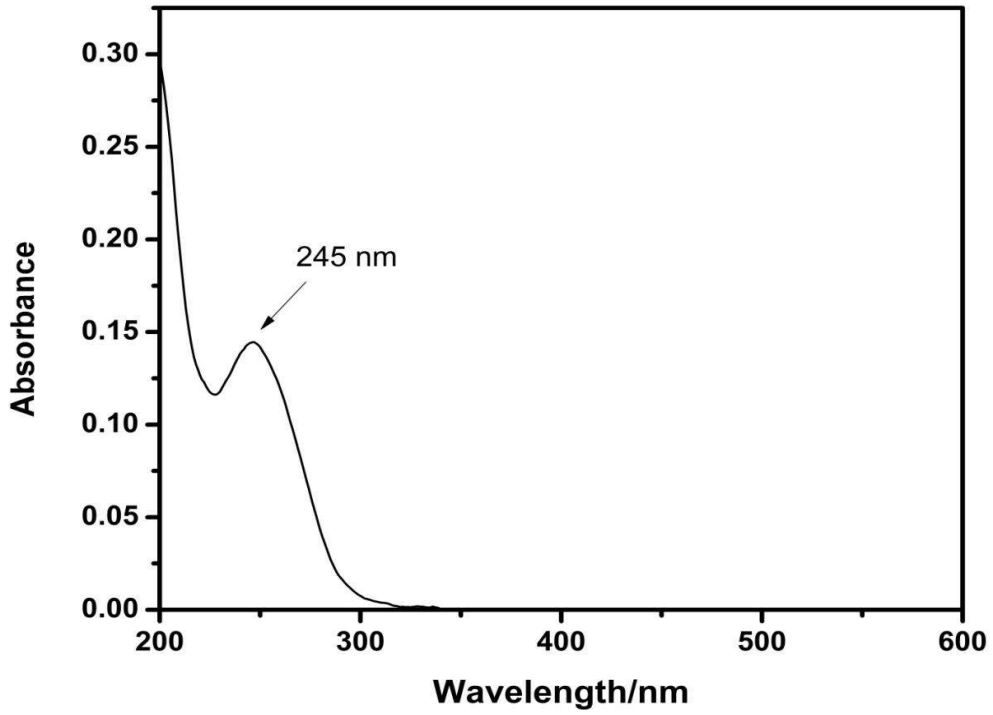

Figure S5: ***UV-VIS spectrum of L2 in MeOH***

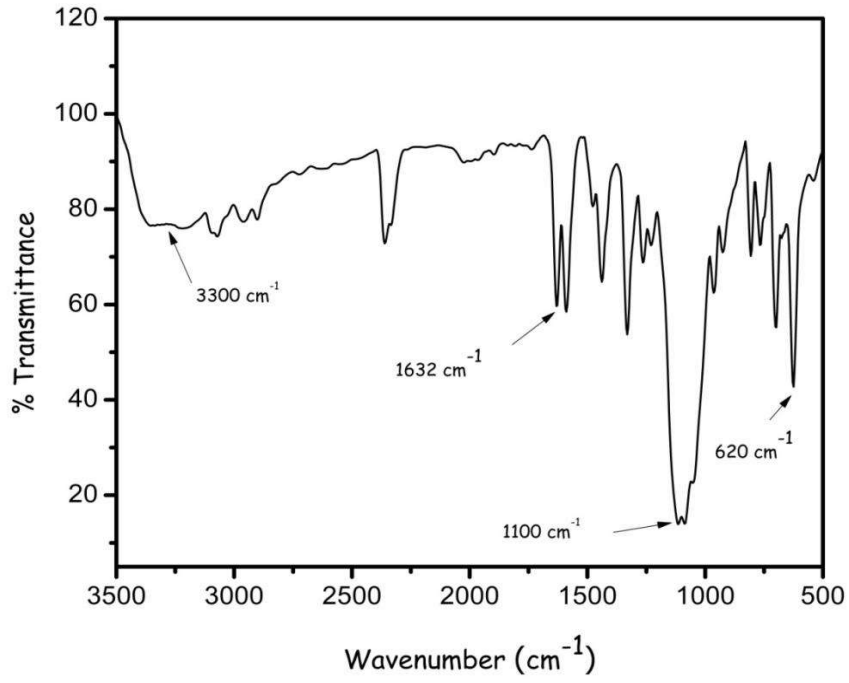

Figure S6: ***IR spectrum of 2***

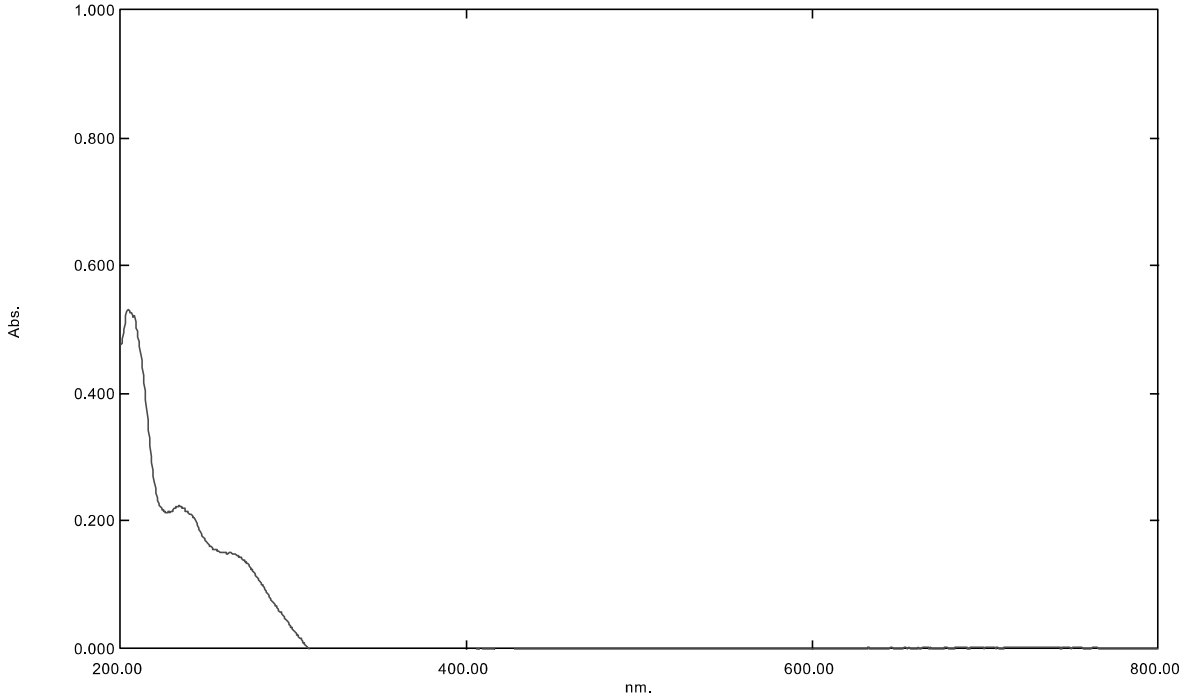

Figure S7: ***UV-VIS spectrum of 2 in MeOH***