Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 10, 2911-2923

Kinetic Study Of Novel Metal- Nucleobase Complex For Phenol Oxidation Enzyme-Mimicking Activity

Srimanta Kumar Das [1*], Biswaranjan Behera [2]

[1] Ph D Scholar,The Pharmaceutical College, Barpali (BPUT,Odisha)India
[2]Department of Pharmacognosy,Kanaka Manjari Institute of Pharmaceutical Sciences, Chhend Rourkela,,Pin-769015 (Odisha)India
**E-mail : rrlsrimanta@gmail.com*

ABSTRACT

Nucleobase Adenine analogues are employed as various therapeutic Medicinal agents. We preapared a novel copper metalated Cu (II) complex of modified Adenine by synthesizing N7 alkyl adenine and metalizing it with Cu (II) salt. This complex catalyses the oxidation of phenolic substrates like catechol, 4-hydroxy anisole, and 4-t-butyl catechol. Because the modified ligand complex is insoluble with general organic solvents, heterogeneous catalytic behaviour, it may be reusable. A Michaelis-Menten kinetic analysis was conducted to compare the catalytic oxidation of metal nucleobase complexes with substrates to that of mushroom tyrosinase. The catalytic oxidation of metal nucleobase complexes with substrates was explored. It was observed that the copper-metalated adenine complex exhibited phenol oxidation activity and enzyme mimicking activity similar to tyrosinase.

Keywords: N7 alkyl adenine Cu (II) complex,phenol oxidation,tyrosinase,catalysis,enzyme mimic activity

INTRODUCTION

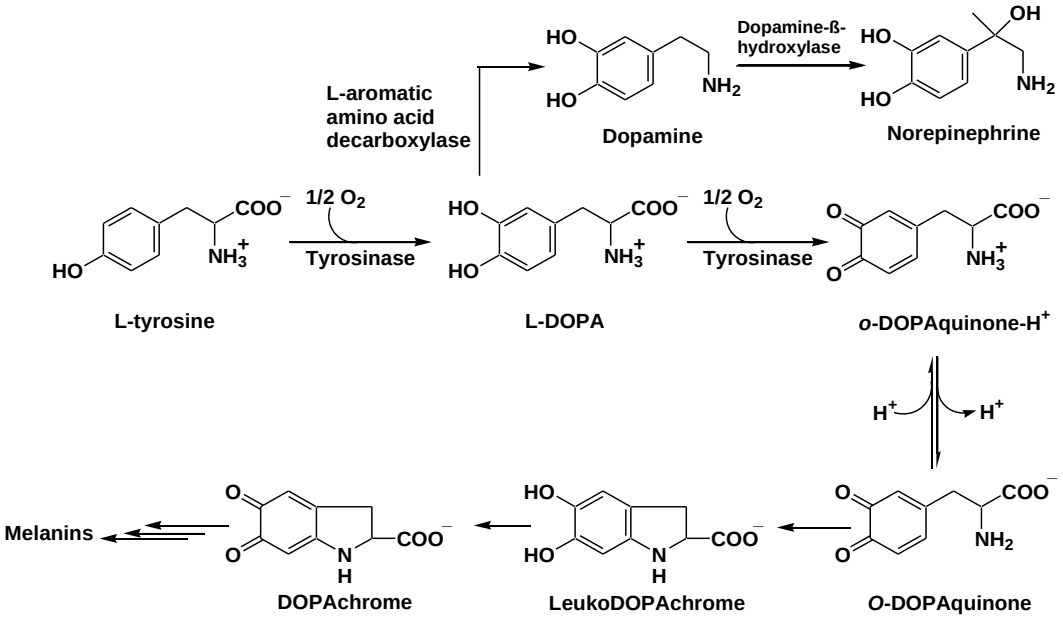
Small molecule modeling of metalloenzymes remains a particularly interesting interdisciplinary research area .The field of biomimetic enzyme catalysis has recently been expanded to include various oxidative transformations of fundamental relevance, which are facilitated by galactose oxidase,[1-4] cytochrome P450[5-7], cytochrome c oxidase,[8-10] catechol oxidases, and tyrosinases.[11-13]. In biological systems metal-containing proteins, copper is involved in oxidation reactions. Tyrosinase, catechol oxidase, and hemocyanin are the three polyphenol oxidases with dinuclear copper centres in their active sites[14-17]For the tyrosinase enzymatic reaction, artificial approaches invariably invoke copper coordination ability of a suitably designed ligand that allows facile activation of molecular oxygen or related species.

A dicopper oxidase with both monophenolase and diphenolase activity is shown by tyrosinase enzyme (EC 1.14.18.1).[18]This enzyme is thought to be involved in the production of the neurotransmitters catecholamine, melanin, and tyrosine (Scheme-1).[19] Numerous small molecule tyrosinase active site models have been described in the literature[20-24], and kinetic investigations of the enzyme's separate catalase and peroxygenase activity components have been published.[25]

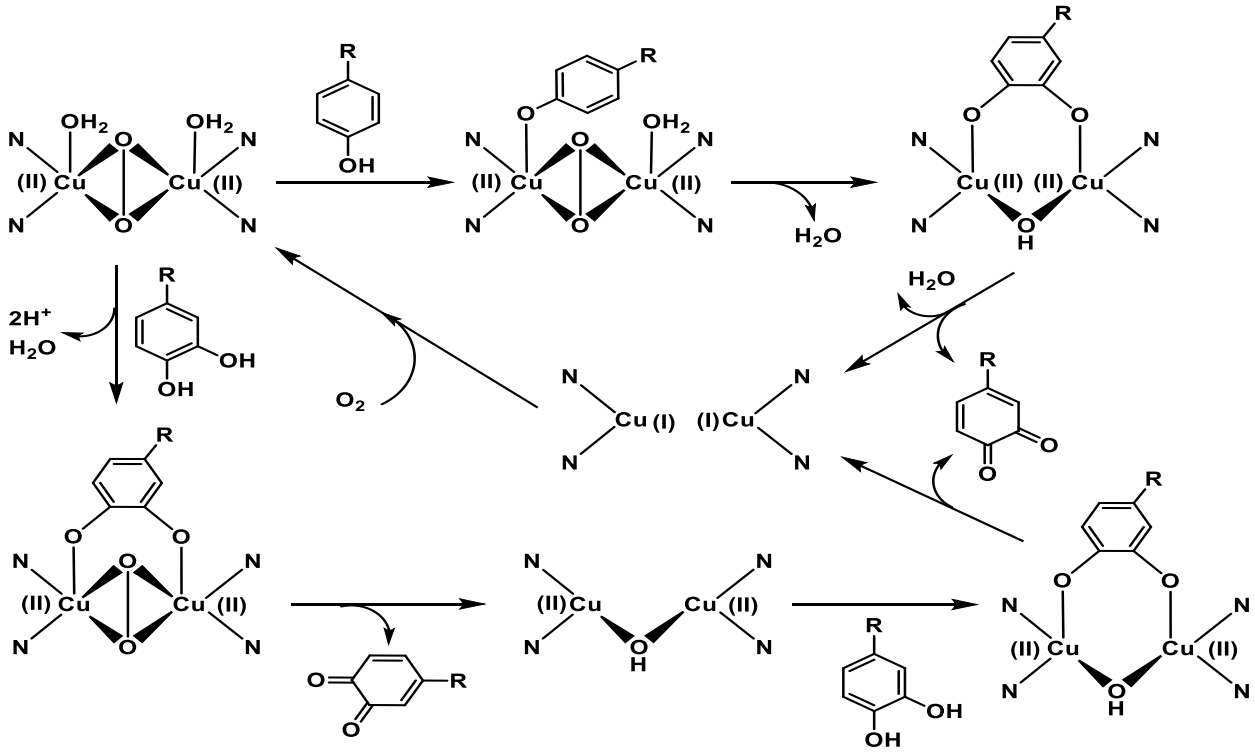
Tyrosine is o-hydroxylated to produce DOPA, which quickly undergoes oxidation to produce o-

DOPA quinone, which is an indication of tyrosinase enzyme activity. (Scheme-1). For melanogenesis[26], where the ortho quinone product undergoes auto polymerization to give a coloured poly phenolic pigments called melanin, generally found in animals, plants, fungi, and bacteria [27], this reaction is extremely essential.

The general approach for creating these enzyme mimics involves positioning two copper atoms closer so that to permit contact with molecular oxygen, which activates oxygen and is necessary for the action of tyrosinase and are summarised this enzyme's reaction pathway in Scheme-2



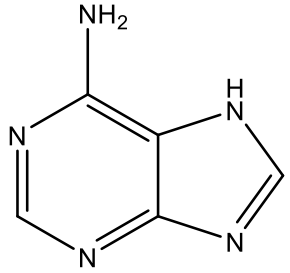
Scheme-1: Biosynthesis of catecholamine neurotransmitters and melanogenesis pathway.



Scheme-2: Catalytic oxidation of mono- and diphenols by tyrosinase

As the enzymes are macromolecular nature, it is possible to imagine metal ligand complex as suitable scaffolds to mimic catalytic activity of enzymes in conjunction with added metal ions. Towards this goal we have synthesized and studied a substituted adenine containing Cu(II) metal complex, which is capable of performing transformations to attain functional mimicry of enzymes for oxygen insertion reactions.[28]We have exploited metal ion coordination ability of natural nucleobase ligands with metal complex to generate a constellation of metal centers for facile catalysis of synthetically useful and biochemically interesting reactions.[28,29]

To explore further and extend the area of this, we choose to work with a purine nucleobase, namely Adenine (Fig-1) for its versatile behavior [30-34].



adenine

Fig-1.Adenine nucleobase structure

After suitable modification and metal complexation. We planned to study the reactivity of this metalated complex and its application in oxidative transformation of phenols. To achieve this, , we synthesized N7 alkylated adenine and was metalated with copper (II)salt and used as catalyst for oxygen insertion (activation) reaction/coupling of phenols, in the absence of added oxidants.

EXPERIMENTAL

Reagents and enzyme

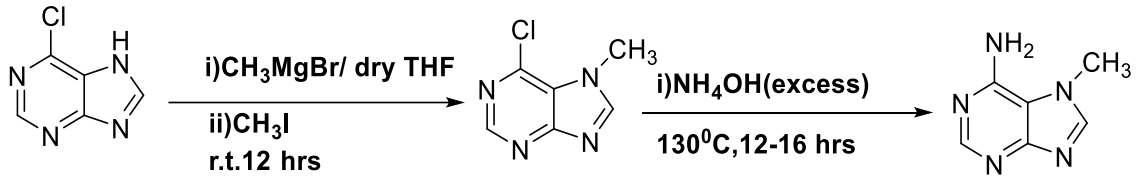
6-chloro purine and copper chlorate hexa hydrate was purchased from S.D.Fine Chemicals, (MBTH) 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (HIMEDIA, India) and 4-t-butylcatechol from Loba Chemicals, India were used. Catechol (S.D. Fine Chemicals, India) and 4-hydroxyanisole (Loba Chemicals, India) were purified by sublimation prior to use. Mushroom tyrosinase enzyme (TYR) (EC 1.14.18.1, 1000U/mg,) was purchased from Sigma Aldrich,India. Methyl alcohol from S.D. Fine Chemicals and distilled water was used in all the assays.The reaction kinetics was studied on UV-Visible spectrophotometer (Shimadzu UV-1800).

Preparation of Metal complexes

1. **N-7-Methyl adenine:Cu complex**

N-7-Methyl adenine was synthesized as per reported literature[35] (Scheme -3) .Methyl adenine (5mg, 0.033mmol) was dissolved in 8 ml of methanol and was placed in 100ml RB with a magnetic stirring bar and was placed on a stirrer. Copper perchlorate hexahydrate Cu $(ClO_4)_2.6H_2O$ (6.11mg, 0.0165 mmol,) in 5ml of methanol was slowly added with continuous stirring. After 5 minutes, a precipitate was formed, which was filtered and washed with methanol several times to remove the unreacted

ligand and metal salt. The product was dried under a high vacuum, and 3.26 mg product was obtained, the complex was extremely low solubility with organic solvent and water, therefore, NMR studies could not be carried out. But, 1mg of the complex was stirred with 5 mL of Methanol, and the supernatant was studied with Mass spectroscopy. The Mass studies revealed that probable Molecular Formula of complex would be $C_{26}H_{36}Cu_2Cl_2N_{20}O_{10}^{2+}$ and Molecular weight 984.09 (m/z = 493.04 expected, Experimental Value m/z = 493.35) was given in scheme-3-and Basing upon the Mass studies, the probable structure of complex as Fig-2 and 3.



.

Scheme-3: Synthesis of **N-7-Methyl adenine**

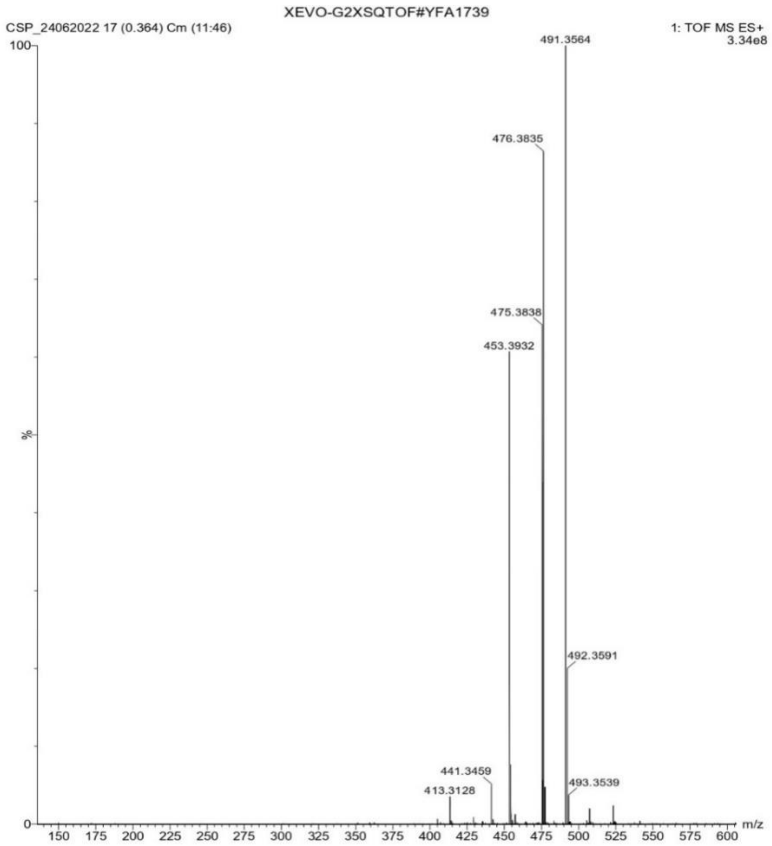


Fig- 2: (Mass spectrum of N-7-Methyl adenine: Cu complex)

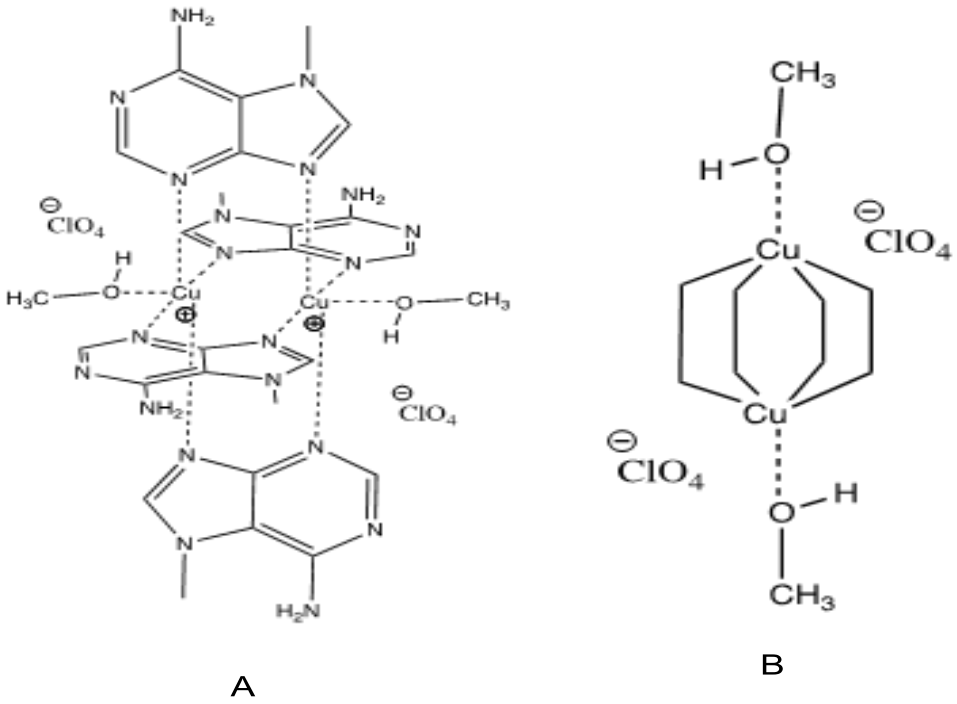


Fig- 3: A) Probable structure of copper complex. B) Cartoon representation of the complex, lines connecting copper centres are the adenine derivative.

Assay of enzymatic phenol oxidation

For kinetic characterizationof phenol oxidation,the spectrophotometric assay was as carried out for coppermetalated AdenineComplex with three substrates (4-hydroxyanisole, catechol, 4-t-butylcatechol). The procedure are as below.

Catechol-tyrosinase oxidation

For oxidation of catechol by tyrosinase, the assay mixture of 3ml was preapared which contained 2.8mL catechol solution (50% aq. Methyl alcohol maintaining pH 7.05 at 20ºC) in various concentrations ranging from 0.5-2.05 mM, 0.1 ml of MBTH in methyl alcohol (1% w/v), and 0.1 mL of mushroom tyrosinase (2.33 U/mL). Due to oxidation reaction in assay mixture,the MBTH-quinone adduct was formed and monitored at λ_{max} 500 nm on UV-Visible spectro photometer (ε= 3.25 x 10^4 M^{-1} cm^{-1}).[36-38] The chromogenic MBTH –quinone adduct with respect to time determined the initial velocities(V) of the reaction and corresponding Lineweaver Burk graph (1/V vs. 1/[S]) was plotted from where Michaelis-Menten parameters were calculated (Fig-4)

Catechol oxidation Assay

The solution of catechol were prepared ranging from concentrations 0.5-2.5mM in 50% aqueous methyl alcohol (pH7.05). From each prepared concentration of 3ml of solution for assay contain 2.8 ml of catechol, and 0.1 mL of MBTH in methyl alcohol (1% w/v), and 0.1ml Cu metalated N7 methyl adenine complex (3 mL/1 mg). The Oxidation reactions were carried in centrifuge glass tubes on a thermostat water bath at 30ºC with continuous stirring for avoiding precipitation of metal complexes. During

oxidation, MBTH-quinone adduct was produced, which was monitored at λ_{max} 500 nm ($\varepsilon = 3.25 \times 10^4\ M^{-1}\ cm^{-1}$)[36-38]. At a regular interval of time the solution absorbance was measured. The chromogenic MBTH –quinone adduct with respect to time determined the initial velocities(V) of the reaction and corresponding Lineweaver Burk graph (1/V vs. 1/[S]) was plotted from where Michaelis-Menten parameters were calculated. (Fig-5)

oxidation of 4-tertiary-butylcatechol Assay

Similarly,The solutions of 4-tert-butylcatechol were prepared ranging from concentrations 0.5-2.5mM in 10% DMF solution in 50% methyl alcohol maintaining with pH 7.05 .From each prepared concentration of 3ml of solution for assay contain 2.8 ml of 4-tert-butylcatechol , 0.1 mL of MBTH (1% w/v in methanol) and 0.1ml of Cu metalated N7 methyl adenine complex (3 mL/1 mg). The Oxidation reactions were carried in centrifuge glass tubes on a thermostat water bath at 30ºC with continuous stirring for avoiding precipitation of metal complexes. During oxidation, MBTH- quinone adduct was produced, which was monitored at λ_{max} 500 nm ($\varepsilon = 3.25 \times 10^4\ M^{-1}\ cm^{-1}$).[36-38] At a regular interval of time the solution absorbance was measured. The chromogenic MBTH –quinone adduct with respect to time determined the initial velocities(V) of the reaction and corresponding Lineweaver Burk graph (1/V vs. 1/[S]) was plotted from where Michaelis-Menten parameters were calculated (Fig-6)

4-Hydroxyanisole oxidation Assay

3ml of 4-Hydroxy Anisole solution assay mixture were prepared, Concentration ranging from (2.0 - 8 mM) were made prepare (50% aq. Methyl alcohol containing 2% DMF) maintaining with pH 7.2. All other conditions were followed same as given above assay procedure for catechol oxidation. The formation of MBTH adduct was monitored at λ_{max} 492nm ($\varepsilon = 3.13 \times 10^4\ M^{-1}\ cm^{-1}$). in UV spectroscopy[20,29,37], Finally, the Michaelis-Menten parameters were calculated from the corresponding Lineweaver-Burk plots (1/V vs. 1/[S]) .(Fig-7)

Reference cell,

The reference cell, which was used for all experiments, comprised 0.1 mL of MBTH in methyl alcohol (1% w/v) and 2.9 mL of substrate without the Cu metalated N7 methyl adenine complex to account for background auto-oxidation. In order to dissolve substrates and the associated MBTH adduct in aqueous methanol, DMF was added as a co-solvent.[20]

RESULTS AND DISCUSSION

Phenol oxidation Kinetics Study:

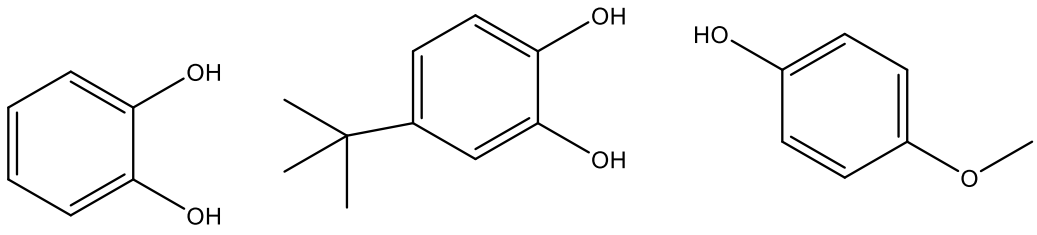
To mimic the catalytic activity of the copper-containing oxidase enzyme tyrosinase was used, and the phenolic substrate were oxidized by using a copper-metalated N7 methyl adenine complex. The conversion of mono- and 1, 2-diphenols to o-quinones is catalysed by this enzyme. Tyrosinase's active site is supported by a binuclear copper core that binds molecular oxygen and activates it for subsequent processes.[38]

Monophenolase or cresolase activity allows it to o-hydroxylate monophenols into o-diphenols and oxidise o-diphenols into o-quinones .Catechol, 4-t-butylcatechol, and 4-hydroxyanisole (Fig-8) were used as three separate monophenolic and diphenolic substrates to test a copper-metalated nucleobase complex for catalytic oxidation activiy.[39-40]

.

In this assay study, we determine the formation of a chromogenic adduct as an indirect method to identify phenol oxidation. We did not attempt to optimisation of chemical reaction or not to isolation of the intermediate oxidation products

because our goal of investigations was to determine the ability of metal complex for oxygen insertion or activation. .

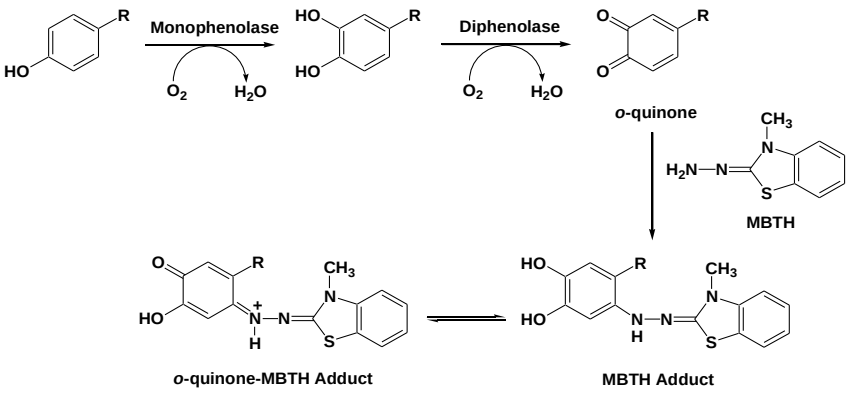


catechol 4-tertiary butyl catechol 4-hydroxy anisole

Fig- 8: catechol, 4-tert-butylcatechol, 4-hydroxyanisole

The generation of colored MBTH adducts (3-methyl-2-benzothiazolinone hydrazonehydrochloride) that produce in the reaction of MBTH with o-quinone was monitored as **Scheme-4.** Our metal complex was insoluble in general solvents, it signify catalytic activity is heterogeneous in nature. The o-quinone formed by the oxidation of substrates (catechol, 4-tert-butylcatechol, and 4-hydroxyanisole) were trapped using MBTH and time-dependent formation of corresponding adducts were measured at respective λmax values (Table1). The Michaelis-Menten parameters were determined for catalytic phenol oxidations from corresponding Lineweaver-Burk plots (1/V vs. 1/[S]) from Figure 4-7 and mention the details on Table-2

.



Scheme-4: Formation o-quinone-MBTH adduct

Table -1: λ_{max} and $\varepsilon_{\lambda max}$ for MBTH adduct

substrate	MBTH adduct λ_{max} (nm)	$\varepsilon_{\lambda max}$ M^{-1} cm^{-1}
Catechol	500	3.25×10^4
4-t-Butylcatechol	494	3.25×10^4
4-Hydroxyanisole	492	3.13×10^4

Table -2: phenol oxidation kinetic parameters by metalated Adenine nucleobase complex

Catalyst	Substrate	K_m (mM)	V_{max} (mM min^{-1})	k_{cat} (min^{-1})
Metal nucleobase complex	Catechol	3.33	25x 10^{-5}	7.9 x 10^{-4}
Metal nucleobase complex	4-t-Butylcatechol	20.88	71.42x 10^{-5}	9.6 x 10^{-5}
Metal nucleobase complex	4-Hydroxyanisole	38.46	33.33x 10^{-5}	1.7 x 10^{-4}
Tyrosinase	catechol	5.0	35.71x10^{-5}	7.7 x10^{-4}

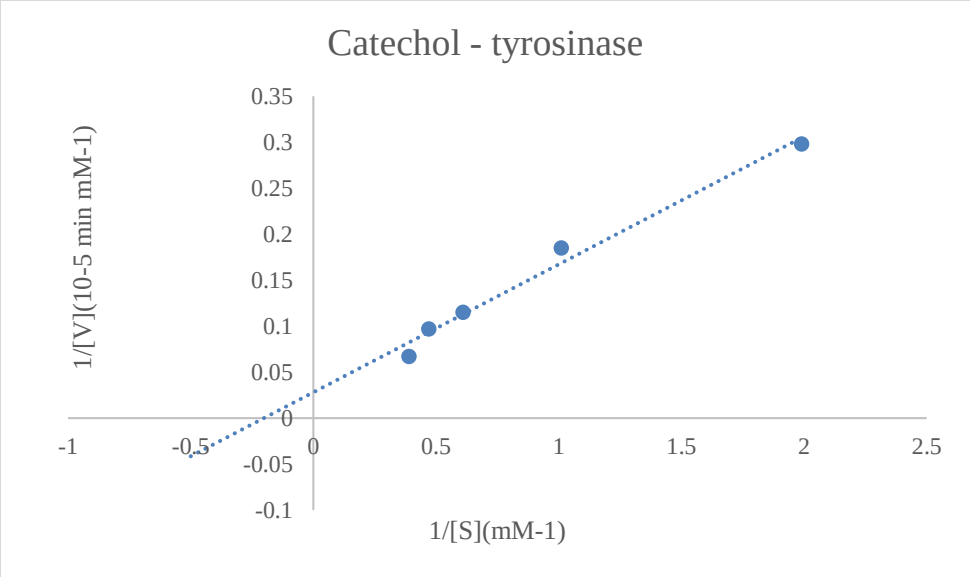


Fig-4: Lineweaver Burk plot for Catechol Oxidation by Tyrosinase

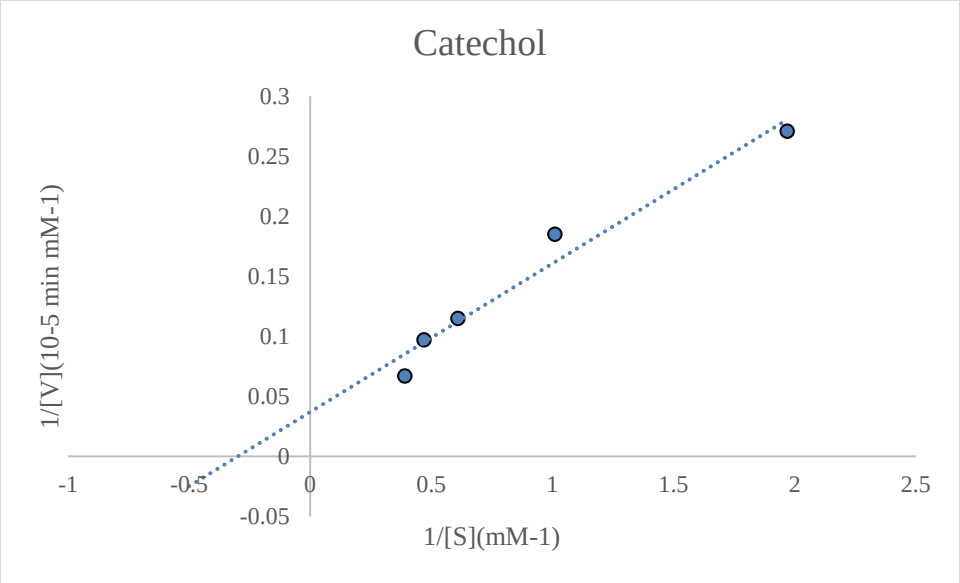


Fig-5 : Lineweaver Burk plot for catechol oxidation

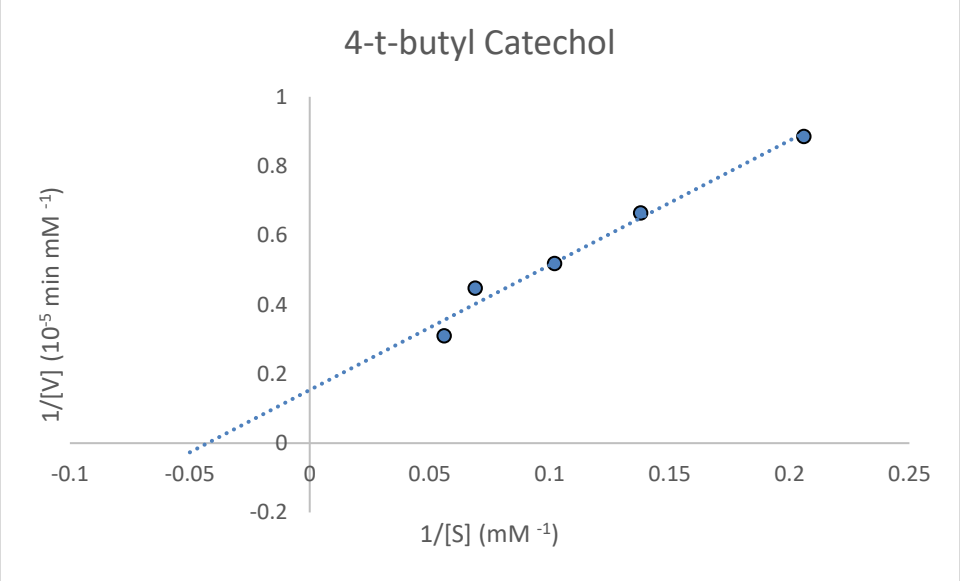


Fig-6. Lineweaver Burk plot for 4-t-butylcatechol oxidation

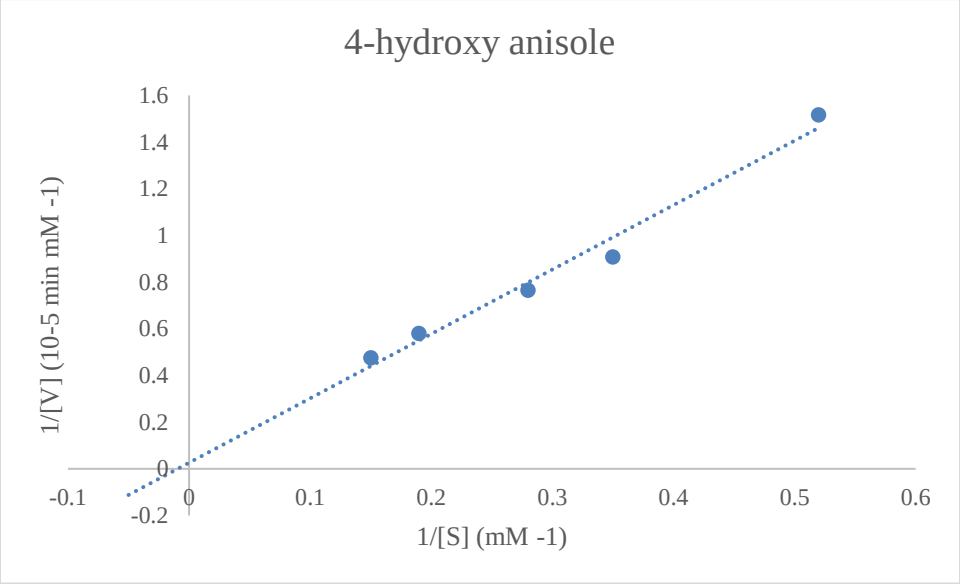


Fig-7. Lineweaver Burk plot for 4-hydroxyanisole oxidation

In this study,the significant monophenol oxidation activity was shown by the metalated adenine complex.The Km, Vmax, and kcat values for monophenol 4-hydroxyanisole were estimated to be 38.46 mM, 33.33x 10^{-5}mM min^{-1},and 1.7 x $10^{-4}min^{-1}$ respectively.

Catechol and 4-tert-butylcatechol substrates were used for the catalysis studies of diphenol oxidation. The Km, Vmax and kcat values for catechol, were found to be 3.33mM, 25×10^{-4} $mMmin^{-1}$, and 7.9×10^{-5} min^{-1}, respectively mM, wheareas 4-t-butylcatechol exhibited Km, Vmax and kcat values as 20.88mM, 71.42×10^{-5} $mMmin^{-1}$, and 9.6×10^{-5} min^{-1}, respectively. The Km and Vmax and Kcat values for the commercial mushroom tyrosinase enzyme was determined using catechol as the substrate and it was found as 5.0 mM, 35.71×10^{-5} $mMmin^{-1}$ and 7.7 x $10^{-4}min^{-1}$, respectively.

Lower Km value of Catechol and 4-tert-butyl catechol as compared to 4-hydroxy anisole,suggested that there is preferential binding of such diphenols to the metalated nucleobase complex.This results are revelead the metal nucleobase complex follow the Michaelis Menton parameter as like tyrosinase and have the catalytic activity.

CONCLUSION

In general Summary, we have studied the kinetic assay of novel Copper metalated N7-Adenine complex ,which has shown tyrosinase like phenol oxidation activity also obeys the Michaelis Menten's kinetics.As the tyrosinase enzyme catalyses the melanogenesis pathway,which increase and decrease level may cause different disorder.So the monitoring of tyrosinase activity is very important and also crucial for development of new therapies.For the development of clinical diagnosis and pharmaceutical drug discovery,the different efficient assays are useful for monitoring tyrosinase activity. This metal complex behaviour just like an small molekule metalloenzyme which mimics the enzyme.It may be useful for the biosynthesis of neurotransmitter like catecholamine, and amino acid containing hydroxyl group.also further poymerisation of complex with a suitable polymer matrix and biopharmaceutics study may useful in the (NDDS) New drug Delivery System.

Researchers have been motivated to create and research smaller synthetic metal complex nucleobases as enzyme mimics by acquiring a fundamental understanding of the mechanism of enzymatic catalysis. Additionally, the study of such synthesis provides a potent tool for the design of novel functional materials, Metal–organic frameworks (MOFs), Bio-MOFs with potential use in molecular, supramolecular, medical science and Pharmaceutical Science.

ACKNOWLEDGEMENT

We are thankful to The Pharmaceutical college,Barpali for providing all facility to carried out the work

REFERENCES

1. Y. Wang, J. L. DuBois, B. Hedman, K. O. Hodgson, T.D. P. Stack, Science, 279, 537-540 **(1998)**
2. F.Michel, F. Thomas, S. Hamman, E. Saint-Aman, C.Bucher, J-L Pierre, Chem. Eur. J., 10, 4115-4125 **(2004)**
3. D.Rokhsana, D. M. Dooley, R. K. Szilagyi, J. Am. Chem.Soc., 128, 15550 -15551 **(2006)**
4. a)H. Oshita, & Y. Shimazaki, Recent Advances in One-Electron-Oxidized CuII–Diphenoxide Chemistry–A European Journal, 26(38), 8324-8340. **(2020)** b). S. P. Meunier,. de Visser, S. Shaik, Chem. Rev. 104, 3947-3980 **(2004)** c) A. K Dhara, K. Kumar, S. Kumari, U. P Singh & K. Ghosh, Transition Metal Chemistry, 45(3), 159-172 **(2020).**
5. D. Kumar, S. P. de Visser, P. K. Sharma, H.Hirao, S. Shaik, Biochemistry, 44, 8148-8158, **(2005)**
6. a)T. L Yusuf, S. D Oladipo, S. Zamisa H. M., Kumalo, Lawal, I. A., Lawal, M. M., & N. Mabuba, ACS omega, 6(21), 13704-13718(2021).. b) J. P. Collman, R. Boulatov, C. J. Sunderland, L. Fu,,Chem. Rev. 104, 561-588 **(2004)** c) B. A., Pandian ,R.. Sathishraj, M. Djanaguiraman, P. V Prasad & M. Jugulam, 9(5), 454 **(2020).**
7. A.Franke, G. Stochel, N. Suzuki, T. Higuchi, K. Okuzono,R. v. Eldik, J. Am. Chem. Soc. 127, 5360-5375 **(2005)**
8. a)S. A., Watson, & G. P McStay,International Journal of Molecular Sciences, 21(19), 7254(**2020).**b) J. P.Collman, N. K. Devaraj, R. A. Decréau, Y. Yang, Y.-L.Yan, W. Ebina, T. A. Eberspacher, C. E. D. Chidsey,Science, 315, 1565-1558**(2007)**
9. W. O. Koch, H.-J. Krüger,Angew. Chem. Int. Ed., 34, 2671-2674 **(2003)**
10. L. M. Mirica, M. Vance, D. J. Rudd,B.Hedman, K. O. Hodgson, E. I. Solomon, T. D. P. Stack, Science 308, 1890-1892**(2005)**
11. a) Y. F., Fan, S. X. Zhu, F. B. Hou, D. F. Zhao, Q. S. Pan, Y. W. Xiang, & P. Wang, , 11(8), 290. **(2021).** b) L. M. Mirica, D. J.Rudd, M. A. Vance, E. I. Solomon, K. O. Hodgson, B.Hedman, T. D. P. Stack, J. Am. Chem. Soc. 128,2654-266 **(2006)**
12. a) M. A. Mc Guirl, D. M. Dooley, Curr. Opin. Chem. Biol. 3, 138-144; (**1999**) b) K. D. Karlin, S. Kaderli, A. D. Zuberbühler, Acc. Chem. Res. 30, 139-147(**1997**) c) W. B. Tolman, Acc. Chem. Res. 30, 227-237(**1997**)
13. a)H. A El-Nashar, M. I. G. El-Din, L. Hritcu, & O. A. Eldahshan, Molecules, 26(24), 7546. (2021) b)B N. C. Eickman, R. S. Himmelwright, E. I. Solomon, Proc. Natl. Acad. Sci. USA, 76, 2094-2098. **(1979).**
14. A. Sánchez-Ferrer, J. N. Rodríguez-López, F. García-Cánovas, F. García-Carmona, Biochim. Biophys. Acta, 1247, 1-11.(**1995)**
15. T. Klabunde, C. Eicken, J. C. Sacchettini, B. Krebs, Nat. Struc. Biol. 5, 1084-1090. **(1998**)
16. E. I. Solomon, U. M. Sundaram, T. E. Machonkin, Chem.Rev. 96, 2563-2606 **(1996)**
17. a) C. Eicken, B. Krebs, J. C. Sacchettini, Curr. Opin.Struct. Biol. 9, 677-683,**(1999)** b)

P. F. Fitzpatrick, Adv.Enzymol. Relat. Areas Mol. Biol. 2000, 74, 235-294; c) F.García-Cánovas, F. García-Carmona, J. Vera, J. L. Iborra,J. A. Lozano, J. Biol. Chem. 257, 8738-8744**(1982)**

18. L. Santagostini, M. Gullotti, E. Monzani, L. Casella, R.Dillinger, F. Tuczek , Chem. Eur. J. 6, 519-522**.(2000)**
19. S. Itoh, H. Kumei, M. Taki, S. Nagatomo, T. Kitagawa,S.Fukuzumi, J. Am. Chem. Soc. 123, 6708-6709 **(2001)**
20. L. M. Mirica, M. Vance, D. J. Rudd, B. Hedman, K. O.Hodgson, E. I. Solomon, T. D. P. Stack, J. Am. Chem.Soc., 124, 9332-9333 (2002)
21. V. Fusi, A. Llobet, J. Mahia, M. Micheloni, P. Paoli, X.Rabas, P. Rossi, Eur. J. Inorg. Chem. 20 02, , 987-990 **(2002)**
22. A. Granata, E. Monzani, L. Casella, J. Biol. Inorg. Chem. 9, 903-913 **(2004)**
23. Y. Hitomi, A. Ando, H. Matsui, T. Ito, T. Tanaka, S.Ogo, T. Funabiki, Inorg. Chem. 44, 3473 -3478. **(2005)**
24. F. García-Cánovas, F. García-Carmona, J. Vera, J. L. Iborra, J. A. Lozano, J. Biol. Chem. 257, 8738-8744 **(1982)**
25. J. M. Pawelek, A. Korner, Am. Sci. 70, 136-145**.(1982)**
26. a) A. Neves, L. M. Rossi, I. Vencato, V. Drago, W. Haase, R. Werner, Inorg. Chim. Acta 281, 111-115 **(1998)** b) D. Ghosh, R. Mukherjee, Inorg. Chem. 37, 6597-6605 **(1998)** c) M. Kodera, H. Shimakoshi, Y. Tachi, K. Katayama, K. Kano, Chem. Lett. 441-442 **(1998)**d) D. A. Rockcliffe, A. E. Martell, Inorg. Chem. 32, 3143-3152 **(1993)**
27. a) R. Gupta, R. Mukherjee, Tetrahedron. Lett. 41, 7763-7767 **(2000)** b) A. Llobet, A. E. Martell, M. A. Martínez, J. Mol. Cat. A: Chem. 129, 19-26 **(1998)** c) R. Gupta, R. Mukherjee, Inorg. Chim. Acta 263, 133-137 **(1997)** d) S. Mahapatra, J. A. Halfen, E. C. Wilkinson, L. Que, Jr., W. B. Tolman, J. Am. Chem. Soc. 116, 9785-9786 **(1994)**
28. S. G. Srivatsan, S. Verma, Chem. Commun. 515- 516 **(2000)** b) S. G. Srivatsan, S. Verma, Chem. Eur. J. 7, 828-833 **(2001)** c) C. Madhavaiah, S. G. Srivatsan, S. Verma, Catal. Commun. 2, 95-99 **(2001)** d) S. G. Srivatsan, M. Parvez, S. Verma, Chem. Eur. J., 8, 5184-5191 **(2002)**
29. S. G. Srivatsan, P. Nigam, M. S. Rao, S. Verma, Appl. Catalytic cytosine polymer: DNA scission and phenol oxidation 156 Catal. A: General 209, 327-334 **(2001)**
30. R. K. Gupta, M., Riaz, M., Ashafaq, Z. Y., R. S., Gao, Li, D. C. Varma & D. Sun, Coordination Chemistry Reviews, 464, 214558. **(2022).**
31. a**)** B. Lippert Coord Chem Rev 200:487–516 **,(2000)** b) B. Lippert Prog Inorg Chem 37:1–97,**(2009)**
32. H. M Hasan, A. I Yahiya, S. S. Hassan, & M. M. Salama, Biological Study of Transition Metal Complexes with Adenine Ligand. Multidisciplinary Digital Publishing Institute Proceedings, 41(1), 77 **(2019).**
33. S., Abdullah, M. S Rahman, H., Yeasmin, A. A., Shaikh, & P. K. Bakshi,. Synthesis and Characterization of Mixed Ligand Complexes of Copper (II) with Adenine and Dicarboxylic Acids. Dhaka University Journal of Science, 69(2), 76-81 **(2021).**
34. M. E. García-Rubiño, A. Matilla-Hernández, A. Frontera, L. Lezama, J. Niclós-Gutiérrez, , & D. Choquesillo-Lazarte, Pharmaceuticals, 14(5), 426 **(2021).**
35. S. Chen, R. F. Graceffa, and A. A. Boezio ,Org. Lett. 18, 16−19 **(2016)**
36. J. C. Espín, M. Morales, R. Varón, J. Tudela, F. García-Cánovas, Anal. Biochem. 231, 237-246.**(1995**)
37. C. S. Purohit a, M. Parvez , S. Verma, Applied Catalysis A: General 316 100–106 **(2007)**
38. J. C. Espín, J. Tudela, F. García-Cánovas, Anal. Biochem. 259, 118-126.(**1998**)

39. J. M. Pawelek, A. Korner, Am. Sci. 70, 136-145(**1982**)
40. A. P. Chaudhuri, M. Hess, T. Weyhermuller, K. Wieghardt,Angew. Chem. Int. Ed. 38, 1095-1098. **(1999)**