J. Mater. Environ. Sci. 6 (8) (2015) 2166-2173
ISSN : 2028-2508
CODEN: JMESCN

Catecholase and tyrosinase biomimetic activities for heteroatom donor ligands: Influence of five parameters

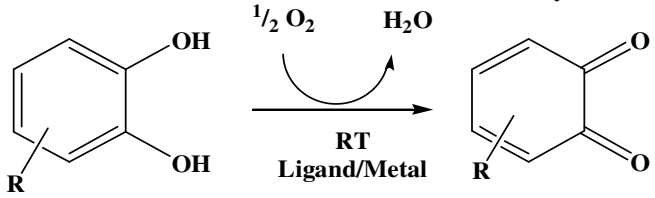
A. Mouadili[1], A. El Ouafi[2], A. Attayibat[1], S. Radi[1], R. Touzani[1,3*]
[1]*LCAE, COSTE, Faculté des Sciences, Université Mohammed Premier, BP 524,60000 Oujda, Morocco.*
[2]*Laboratoire d'Analyses Médicales, CHP, Berkane, Morocco.*
[3]*Faculté Pluridisciplinaire de Nador, Université Mohammed Premier, BP 300, Selouane 62700, Nador, Morocco.*

Received 01 June 2015, Revised 22 July 2015; Accepted 22 July 2015.
**Corresponding authors: E-mail: touzanir@yahoo.fr* (+ 212 677 968 240).

Abstract
The *in-situ* transition metal complexes of five ligands **L_1-L_5** were reported and examined for their catecholase activities at ambient conditions, we found that reaction rate depends on five parameters: Nature of the ligand; nature of metal salts, concentration of the combination of metal/ligand; nature of solvent and nature of the substrate. The highest rate activity is given by the *in-situ* complex formed by ligand **L_1** and metal salt **$Cu(CH_3CO_2)_2$** which is equal to 19.04 µmol.$L^{-1}$$min^{-1}$.

Keywords: pyrazol, catechol, heteroatom compound, transition metal; oxidation reaction.

1. Introduction
The active sites of proteins / enzymes mediate still playing an important scope of chemical processes including electron transfer, reversible dioxygen binding and activation, and nitrogen oxide transformations [1-2]. Biological binuclear copper centres' involved with dioxygen binding and activation includes hemocyanins (Hcs), tyrosinase (Tyr), and catechol oxidase (CO) [3-6]. Notable advances in the understanding of the properties of these proteins have been achieved through the comparison of the biomimetic inorganic model studies [4, 5]. The catechol derivatives are common substrates in CO enzyme research [3-6] (**Figure 1**).



catechol — ***o*-quinone**
R = H; ^{t}-Bu; NO_2 — 390- 400 nm

Ligands : **L_1-L_5**
Metals : $Cu(CH_3CO_2)_2$; $Cu(NO_3)_2$; $CuSO_4$; $CuCl_2$; $NiCl_2$; $CoCl_2$; $ZnCl_2$.

Figure 1: reaction model for CO investigation

The coordination of the catechol to the metal centers has been suggested to favor the intermolecular electron transfer reaction that results in the release of *o*-quinone. This could then react with O_2 to restore the active form of the enzyme [3]. It was observed that the catalytic activities of the complexes are not only dependent on the organic ligand but also on the type of inorganic anion coordinated to copper center [7]. Several papers have been published concerning binuclear copper complexes, as models for catechol oxidase (CO) [8-11]. With our own research program in synthesizing pyrazolyl as nitrogen donor ligand and their starting materials [12] we have also been interested in studying their CO proprieties, while CO converts catechols to *o*-quinones [13-24]. Herein we describe the catecholase activity using **L_1-L_5** ligands, and the evaluation of the influence of the structure of ligands, solvent, concentration and substrate towards CO of catechol derivatives to *o*-quinone in an attempt to model the activity of the copper containing enzyme tyrosinase.

2. Materials and methods

2.1. Chemistry:

Pyrazolyl derivative ligands **L_1.L_2** and dicarbonyle compounds **L_3-L_5** (Figure 2) were known products [25].

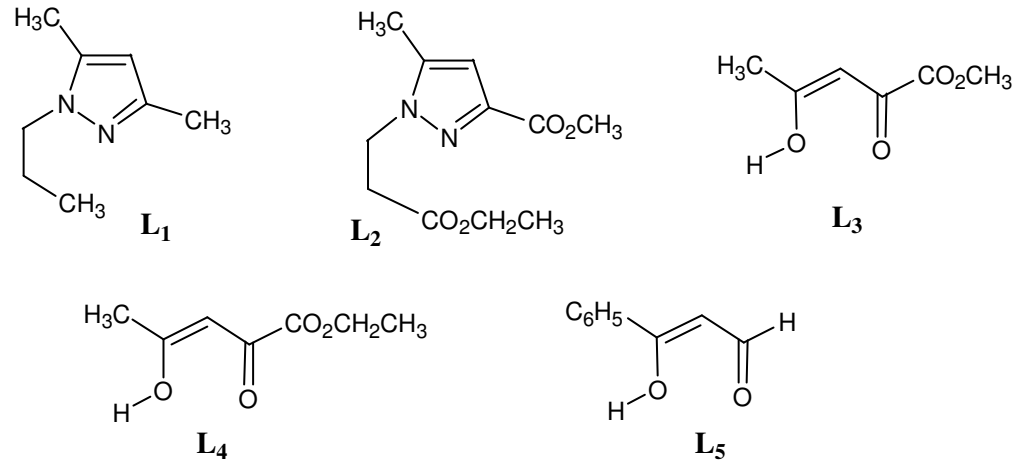


Figure 2: Structure of ligands

2.2. Experimental section, Catecholase Activity Measurements

Kinetic measurements were made spectrophotometrically on UV-Visible Cecil CE 292 Digital Spectrophotometer, following the appearance of *o*-quinones over time at 25°C (390-400 nm absorbance maximum, ε = 1600 $M^{-1}cm^{-1}$ in methanol; ε = 1900 M^{-1} cm^{-1} in THF and ε = 1600 M^{-1} cm^{-1} in acetonitrile). The metal complex (prepared *in situ* from metal salt and the ligand, 0.3 mL of 10^{-3} M solvent solution) [7] and a 2 mL solution (10^{-1} M methanol solution) of catechol derivatives were added together in the spectrophotometric cell. In all cases, catecholase activity was noted.

3. Result and discussion

3.1. Oxydation of the catechol in presence of L_1-L_5 and different metal salts:

The progress of the catechol oxidation reaction is conveniently followed by monitoring the strong absorbance peak of the *o*-quinone substrate on the UV/Vis spectrophotometer. The metal complex (prepared in *situ* from metal salt and the ligand) [26], and a solution of catechol was added together in the spectrophotometric cell at room temperature. Formation of *o*-quinone was monitored by the increase in absorbance at 390-400 nm as a function of time. In all cases, catecholase activity was noted. **Table 1** shows the activities for the first 60 min of the reaction for the different metal complexes. As you can see in the **Table 1**, all of the complexes of ligands **L_1**-**L_5** catalyze the oxidation reaction of catechol to *o*-quinone with the rate varying from a high of 16.54 µmol.L^{-1}.min^{-1} for the **L_1/[$Cu(CH_3COO)_2$]** complex to a low value of 0.09 µmol.L^{-1}.min^{-1} for **L_5/[$CoCl_2$]** *in-situ* complex. These rates are comparable with those reported by Malachowski et *al.,* [27]. Two factors are responsible: - the nature of the ligand structures, which can facilitate the binding of the catechol in a bridging mode and the two-electron transfer step required in the oxidation process. – The second one is la nature of metal salts from copper to zinc. The order of reactivity for the oxidation of catechol by complexes can be dressed as below **L_1 > L_2 > L_3 > L_4 > L_5**.

Table1: Rate activity for catechol oxidation by L/M complexes in MeOH [µmol.L^{-1}.min^{-1})]

L/M	$Cu(CH_3COO)_2$	$CuSO_4$	$Cu(NO_3)_2$	$CuCl_2$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
L_1	16.54	3.60	1.85	1.42	0.71	0.53	0.55
L_2	14.89	3.60	1.13	1.26	0.33	0.24	1.79
L_3	14.02	2.73	0.88	1.35	0.64	0.26	1.45
L_4	12.13	1.98	0.53	1.07	0.21	0.75	0.79
L_5	4.10	1.54	1.74	0.12	0.09	0.23	1.71

We can say that changing from the pyrazle ring to the dicarbonyle moieties has an effect on the rate of the oxidation reaction activities. As you can see in the **Table1**, the nature of the structure of ligand has a large effect on the rate of the reaction; the catalytic activity of theses complexes dependent on the type of inorganic anion coordinated to metal center.

3.2. Effect of the concentration of ligand and metal salts on the CO activity

A comparison of results shown in **Tables 2-3**, we can see clearly the effect of the concentration on the *in-situ* complexes combination. The percentage of two metals and one ligand confirm the relation between the existences of two copper on the enzymatic catecholase process. The contre anion CH_3COO^- gives the highest oxidation rate with the $\mathbf{L_1}$ (about 19.04 µmol.L^{-1}.min^{-1}).

Table 2: Oxidation rates of catechol (µmol.L^{-1}.min^{-1}) (L/M : 1/2) in MeOH

L/M	$Cu(CH_3COO)_2$	$CuSO_4$	$Cu(NO_3)_2$	$CuCl_2$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
L_1	19.04	3.98	1.98	1.63	1.02	0.43	3.19
L_2	17.49	2.75	1.65	1.28	0.39	0.32	1.81
L_3	14.96	3.13	1.03	1.43	0.61	0.06	1.19
L_4	16.40	1.87	0.64	1.20	0.19	0.86	0.82
L_5	4.20	1.65	1.83	0.12	0.09	0.28	1.93

Table 3: Oxidation rates of catechol (µmol.L^{-1}.min^{-1}) (L/M : 2/1) in MeOH

L/M	$Cu(CH_3COO)_2$	$CuSO_4$	$Cu(NO_3)_2$	$CuCl_2$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
L_1	6.13	1.11	1.33	2.19	0.13	0.21	1.38
L_2	5.66	1.87	1.51	1.53	0.32	0.24	1.84
L_3	5.07	0.84	0.81	1.73	0.42	0.26	1.32
L_4	5.23	0.65	0.37	0.77	0.19	0.92	1.09
L_5	2.67	0.98	0.84	1.91	0.06	0.61	1.29

3.3. Effect of solvent

We chose three solvents to prove the effect of the media in such reactions (MeOH, THF and Acetonitrile) **Figure 3**:

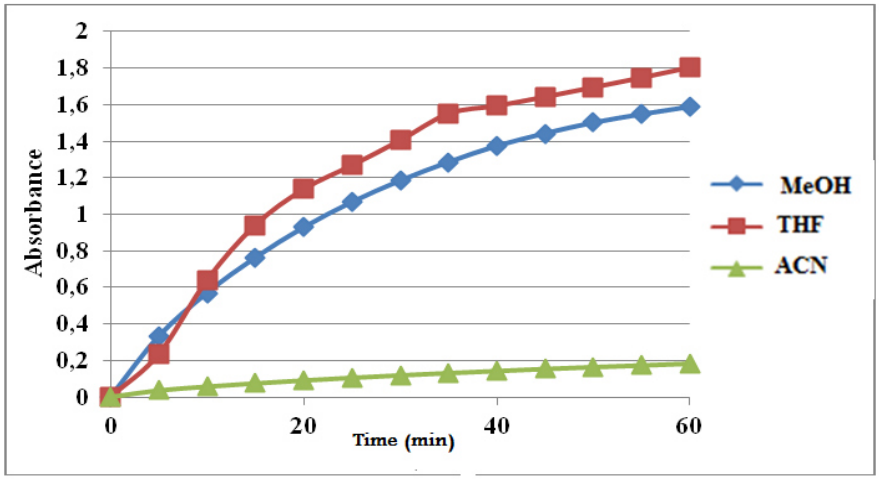
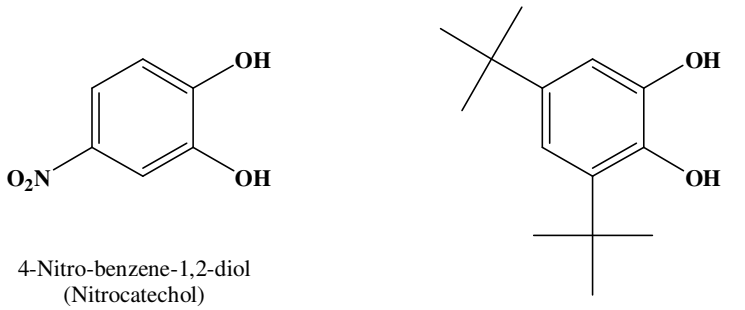


Figure 3: Catechol oxidation in presence of $\mathbf{L_1/Cu(CH_3COO)_2}$ in different solvents

As you can see from the **Figure 3**, the nature of solvent has a huge effect on the catechol oxidation rates. We can conclude that THF (18.75 µmol.L^{-1}.min^{-1}) is the good solvent for this reaction with the combination $\mathbf{L_1/(Cu(CH_3COO)_2}$, Followed by MeOH (16.54 µmol.L^{-1}.min^{-1}) then the acetonitrile (2.05µmol.L^{-1}.min^{-1}). Du may be to the polarity of the solvents.

3.4. Effect of substrate

We have chosen two other substrates to show the influence of this moiety on the catechol oxidation rate (**Figure 4**).



4-Nitro-benzene-1,2-diol
(Nitrocatechol)

3,5-Di-*tert*-butyl-benzene-1,2-diol
(Di-*tert*-butyl-Catechol)

Figure 4: Two other tested substrates

Table 4: Oxidation rates of nitrocatechol in MeOH ($\mu mol.L^{-1}.min^{-1}$)

L/M	$Cu(CH_3COO)_2$	$CuSO_4$	$Cu(NO_3)_2$	$CuCl_2$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
L_1	2.10	1.92	1.99	1.51	0.92	0.53	0.59
L_2	1.87	0.85	0.79	0.96	0.81	0.77	0.81
L_3	2.15	1.83	1.82	1.76	0.39	0.26	0.09
L_4	2.01	1.54	1.42	0.82	0.16	0.09	1.04
L_5	4.04	1.07	2.12	0.63	0.08	0.08	0.08

Table 5: Oxidation rates of 3,5-DTBC in MeOH ($\mu mol.L^{-1}.min^{-1}$)

L/M	$Cu(CH_3COO)_2$	$CuSO_4$	$Cu(NO_3)_2$	$CuCl_2$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
L_1	12.28	4.87	8.72	4.88	0.06	0.48	1.04
L_2	12.57	5.28	2.02	2.29	0.06	1.79	1.79
L_3	12.11	2.57	4.13	1.70	3.36	1.71	1.99
L_4	9.25	8.27	2.13	1.05	0.21	0.99	0.14
L_5	4 .11	3.89	3.45	3.43	0.19	0.91	0.05

As you can see from the **Tables 4- 5**, the substrate has huge importance on the oxidation rates using the same in-situ prepared complexes we can conclude for the combination $L_1/Cu(CH_3COO)_2$ this order : catechol (16.54 $\mu mol.L^{-1}.min^{-1}$) > 3,5-DTBC (12.57 $\mu mol.L^{-1}.min^{-1}$) > nitrocatechol (2.10 $\mu mol.L^{-1}.min^{-1}$).

3.5. Kenitic studies:

3.5.1. Catechole oxidation

To gain better understand of the influence of solvent on the oxidation rates of catechol, we carried the following study. Kinetic study determined by the initial rate method was performed with the best catalysts [$L_1/Cu(CH_3CO_2)_2$], in booth MeOH and THF solvents (**Figure 5**). Solutions containing different concentrations of substrate were prepared from a concentrated stock solution. To determine the dependence of the rates on the substrate concentration, solutions of *in situ* generated complex were treated with increasing amounts of catechol. Initial rates were determined from the slope of the tangent of the absorbance vs time curve after the induction period of 20 min (**Figures 5**). The parameters that we have determined are K_M and V_{max} [28]. The Michaelis kinetic parameter K_M represents the dissociation constant of the intermediate compound *catechol-Cat* (**Figures 6-7**). More K_M is smaller; more the catalyst has the affinity for catechol substrate. V_{max} corresponds to the maximum initial rate of reaction when the catalyst is linked to the substrate. The experimental kinetic parameters are presented in **Table 6**.

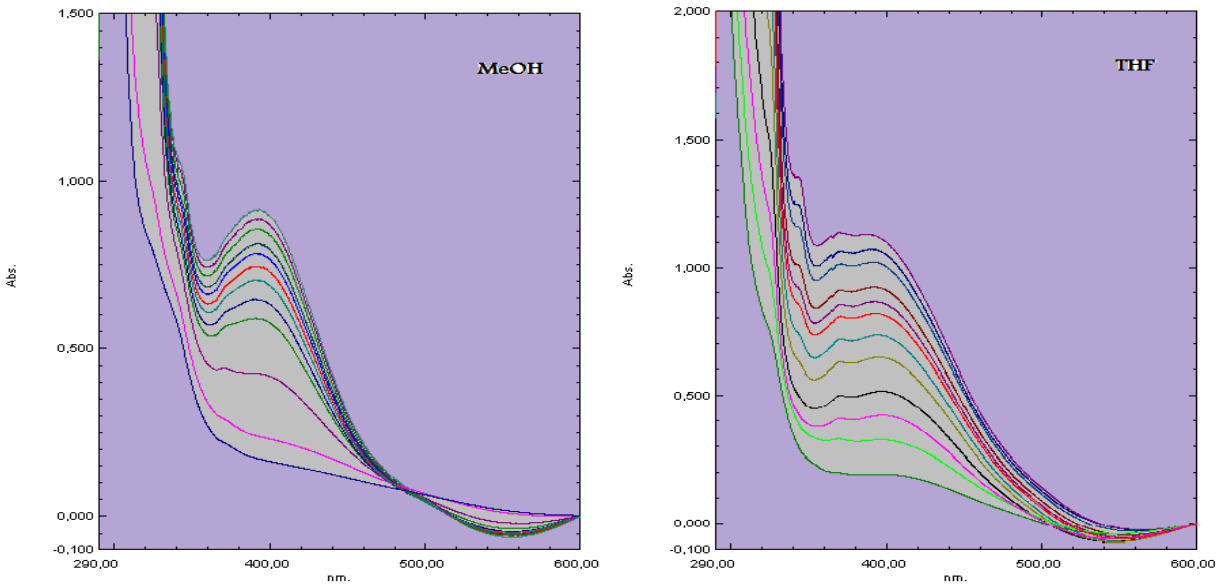


Figure 5: Absorbance of the *o*-quinone using **L_1/Cu(CH_3COO)$_2$** in different solvent

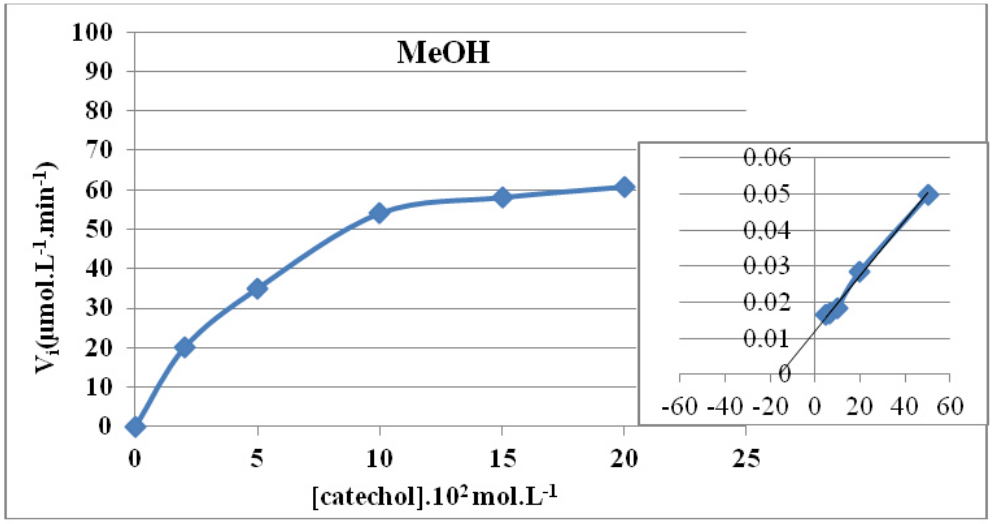


Figure 6: Reaction dependence on the concentration of catechol using **L_1/[Cu(CH_3COO)$_2$]** in MeOH

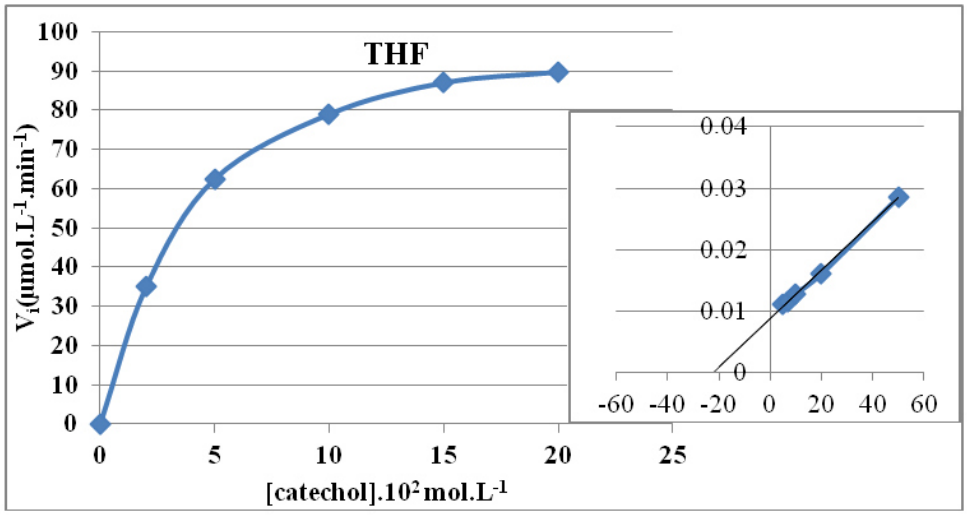


Figure 7: Reaction dependence on the concentration of catechol using **L_1/[Cu(CH_3COO)$_2$]** in THF

Table 6: Kenitic parameters of the oxidation of catechol using $L_1/Cu(CH_3COO)_2$ in MeOH and THF

	MeOH	THF
V_m (µmol.L^{-1}.min^{-1})	60.67	89.72
K_M(mol.L^{-1})	0.04	0.03

3.5.2. Oxidation of 3-5-Di-tert-butylcatechol:

A study by the catecholase activity was performed using 3,5-di-tert-butyl catechol (3,5-DTBC) as substrate by recording the band 'absorption for each 15 minutes of oxidation reaction of 3 , 5-Di-tert-butylcatechol in the presence of combinations **L_1 / $Cu(CH_3COO)_2$** in methanol, the obtained results are in **Figure 8.**

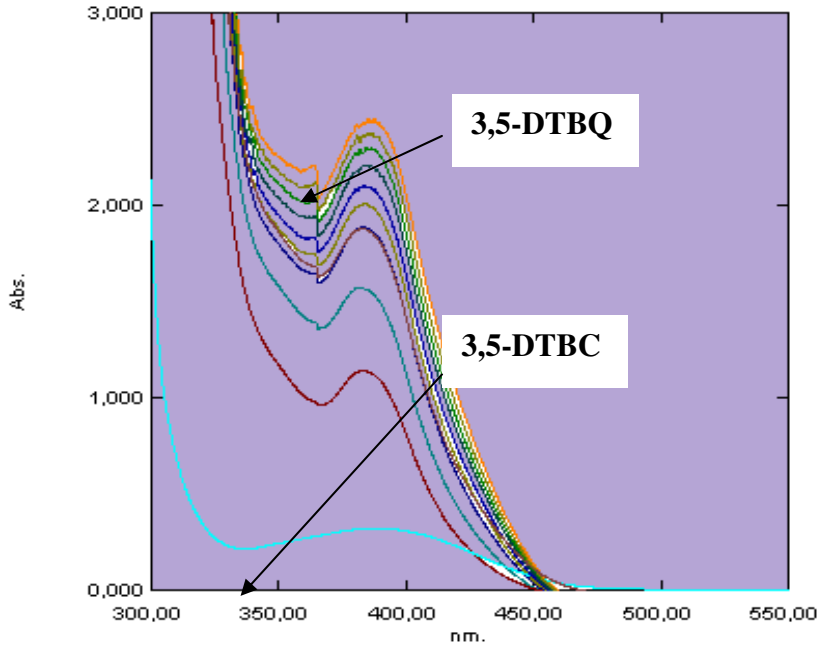


Figure 8: Absorbance of the oxidation of 3,5-DTBC using **$L_1/Cu(CH_3COO)_2$** in MeOH.

3.6. Oxidation of phenol: tyrosinase activities

We have tested these combinations for tyrosinase activity (oxidation of phenol to catechol) (**Figure 9**) [29-31]. This reaction was performed by successively mixing equivalent amounts of a solution (2.10^{-3} mol /L) metal salt of $Cu(CH_3COO)_2$ and of a solution (2.10^{-3} mol/L) ligand, then added 2 mL of phenol (10^{-1}M).

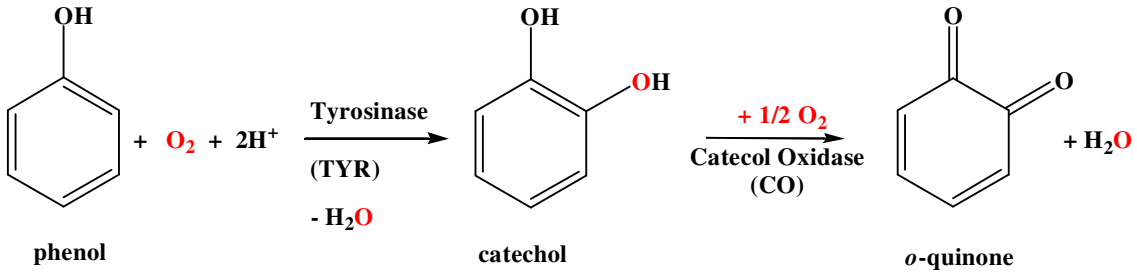


Figure 9: Tyrosinase reaction model

We have found that ligands **L_1, L_2, L_5** have no tyrosinase activity against the two other ligands **L_3** and **L_4** have different catalytic activities on the oxidation of phenol. Recording the absorbance spectrum of evolution of *o*-quinone versus times every 10 min for **L_3-L_4** ligands, and we obtained the following spectra (**Figures 10-11**).
The appearance of the band at 390 nm indicates the formation of *o*-quinone, which explains that phenol is transformed into quinone after the formation of catechol which shows that our suits have dual activities: catecholase activity and tyrosinase activity, as two figures above (**Figures 10-11**). The *o*-quinone absorbance band is wider when using a combination based ligand **L_3**, and **L_4** based combination has a low absorption band

under the same conditions of the oxidation reaction, which confirms that the tyrosinase activity is influenced by the nature of ligands.

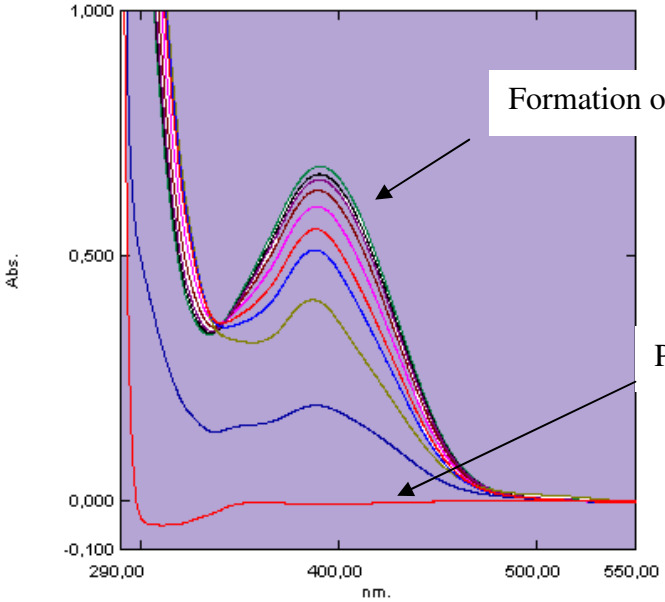


Fig.10: Absorbance spectra showing tyrosinase activity of $\mathbf{L_3/Cu(CH_3COO)_2}$ in MeOH

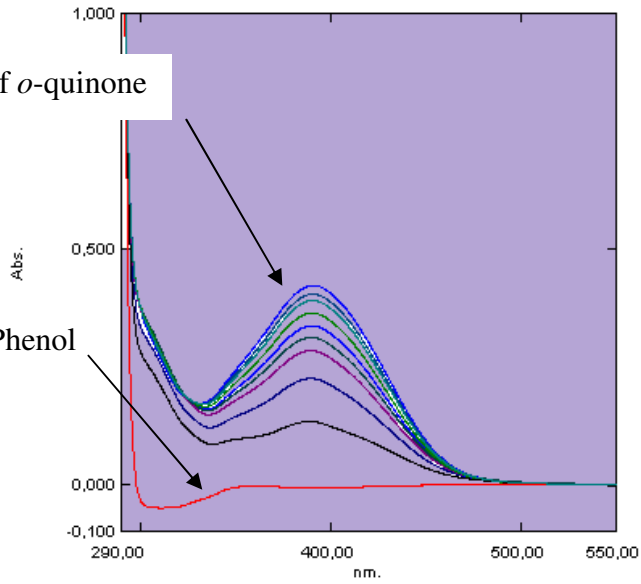


Fig. 11: Absorbance spectra showing tyrosinase activity of $\mathbf{L_4/Cu(CH_3COO)_2}$ in MeOH

4. Conclusion

Five ligands $\mathbf{L_1}$-$\mathbf{L_5}$, were reported and examined for their catecholase activities at ambient conditions. The metallic complexes formed *in-situ* from $\mathbf{L_1}$-$\mathbf{L_5}$ and $\mathbf{Cu(CH_3COO)_2}$, $\mathbf{CuSO_4}$, $\mathbf{Cu(NO_3)_2}$, $\mathbf{CuCl_2}$, $\mathbf{CoCl_2}$, $\mathbf{NiCl_2}$ and $\mathbf{ZnCl_2}$ show significant catalytic influence pathway on the oxidation of catechol and 3,5-DTBC to the corresponding *o*-quinones via formation of a dinuclear species. To more understand the parameters influencing the catalytic activity of the studied complexes and to understand the key properties of solvents which have a controlling role in the catecholase activity, the effect of ligand concentration, the nature of substrate, and the effect of the solvent are studied. The reaction follows Michaelis–Menten enzymatic reaction kinetics to determinate the kinetic parameters.

Acknowledgment - The authors are grateful to the UMP-CUD support for the spectrophotometer Uv-Visible, Also the authors want to thank Professors A. Ramdani and S. El Kadiri for the initiation of these studies.

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