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Catecholase activity studies of two multidendate ligands based on pyrazole

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Abstract

Square wave adsorptive stripping voltammetric determinations of trace concentrations of the xanthene coloring agent erythrosine b were described. The analytical methodology used was based on the adsorptive preconcentration of the dye on the hanging mercury drop electrode and then a negative sweep initiated. Hence, in pH 8.5 carbonate supporting electrolyte, erythrosine b gave a well- defined and sensitive AdSV peak at –1000 mV. The electroanalytical determination of this dye was found to be optimized in carbonate buffer (pH 8.5) with the following experimental conditions: accumulation time (150 s), accumulation potential (0.0 V), scan rate (1000 mV s^{-1}), pulse amplitude (100 mV) and frequency (30 Hz). Under these optimized conditions the AdSV peak current was proportional over the concentration range 1×10^{-7}- 2.5×10^{-7} mol l^{-1} (r = 0.99) with a detection limit of 6.96×10^{-10} mol l^{-1}. This analytical approach possessed enhanced sensitivity than the conventional HPLC or spectrophotometry and it was simple and not time-consuming. The precision of the method in terms of RSD%, was 1.7 % whereas the accuracy was evaluated via the mean recovery of 101.8%±1.79. Possible interferences by several substances usually present as food additive azo dyes (E110, E102), allura red, carmine, amaranth, natural and artificial sweeteners, preservers and antioxidant were also investigated. Applicability of the developed electroanalysis method was illustrated via the determination of erythrosine b in soft drink samples.

Keywords: erythrosine b, square wave, adsorptive voltammetry, food dye.

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1. Introduction

The oxidation reaction still playing an important scope in chemical processes including Organic chemistry, Biochemistry, Biology, materials science, physiology, toxicology, immunology, pharmacology, and biodiversity conservation [1-10]. Otherwise, enzymatic biological oxidations involved with dioxygen binding and activation includes hemocyanins (Hcs), tyrosinase (Tyr), and catechol oxidase (CO) [11-14]. Notable advances in the understanding of the properties of these proteins have been achieved through the comparison of the biomimetic inorganic model studies [15, 16]. The catechol was common substrates in CO enzyme research (Figure 1).

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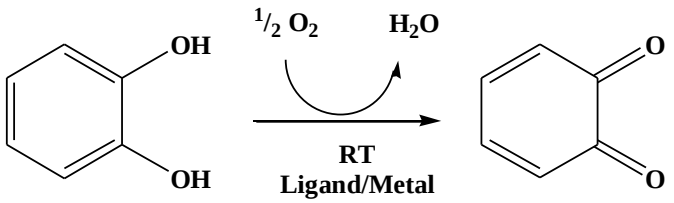


catechol ***o*-quinone**

400 nm

Ligands : L_1-L_2

Metals : $Cu(CH_3CO_2)_2$; $Cu(NO_3)_2$; $CuSO_4$; $CuCl_2$; $NiCl_2$; $CoCl_2$; $ZnCl_2$.

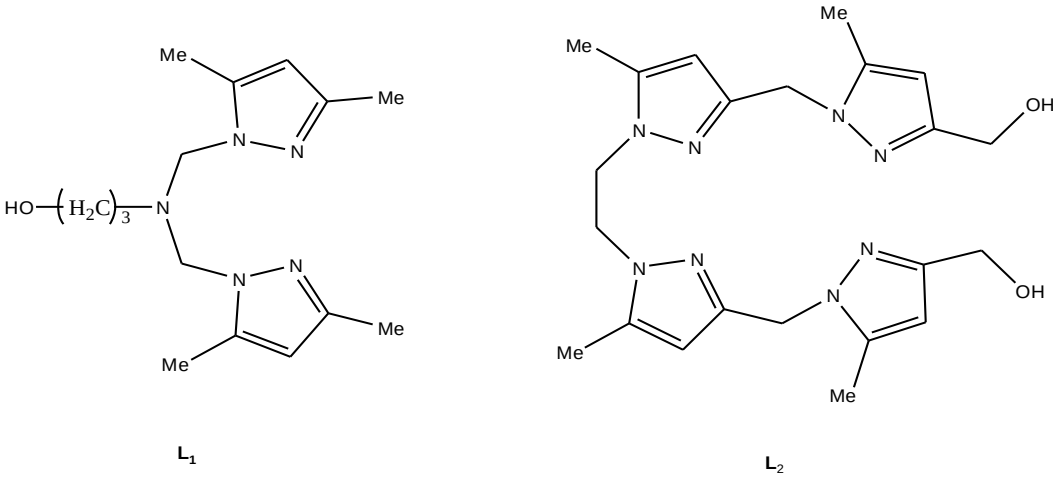
Figure 1: reaction model for CO investigation

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 [11]. 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. Several papers have been published concerning binuclear copper complexes, as models for catechol oxidase (CO). In continuation of our own research program concerning the study of CO proprieties, which converts catechols to *o*-quinones [17-28]. Herein we describe the catecholase activity using L_1-L_2 ligands, and the evaluation of the influence of the structure of ligands, and solvent.

2. Materials and methods

2.1. Chemistry:

Pyrazolyl derivative ligands L_1-L_2 (Figure 2) were known products [29-30].



L_1 L_2

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*-quinone over time at 25°C (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) [11] 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_2 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) [28], 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 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_2** catalyze the oxidation reaction of catechol to *o*-quinone with the rate varying from a high of **19.711** $\mu mol.L^{-1}.min^{-1}$ for the **L_1/[$Cu(CH_3COO)_2$]** complex to a low value of 0.254 $\mu mol.L^{-1}.min^{-1}$ for **L_2/[$CoCl_2$]** *in-situ* complex. These rates are comparable with those reported by Malachowski et *al.*, [31]. 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**.

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

L/M	$Cu(CH_3COO)_2$	$CuSO_4$	$Cu(NO_3)_2$	$CuCl_2$	$NiCl_2$	$Co\ Cl_2$	$ZnCl_2$
L_1	**19.711**	7.773	1.862	0.743	1.601	0.387	2.514
L_2	15.926	2.993	2.175	2.612	0.668	0.254	3.046

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 Figures 3-6, 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.

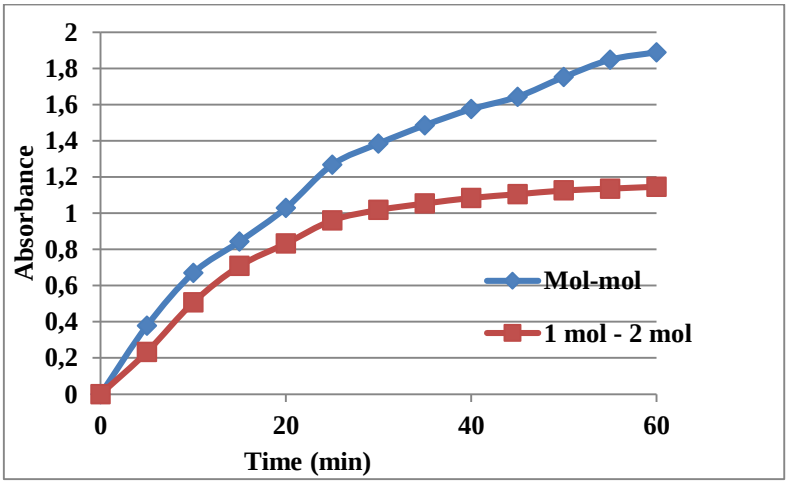


Figure 3: Oxidation of catechol in presence of **[L_1/** $Cu(CH_3COO)_2$] (2/1) in MeOH

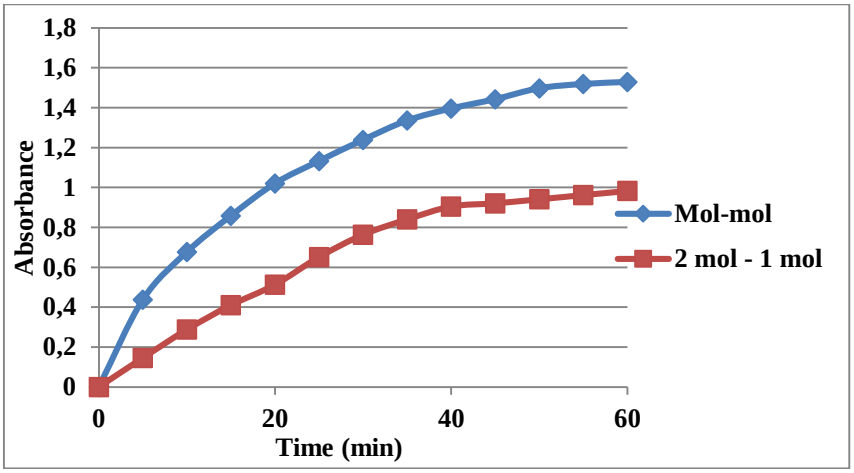


Figure 4: Oxidation of catechol in presence of **[L**$_2$/ Cu(CH$_3$COO)$_2$] (2/1) in MeOH

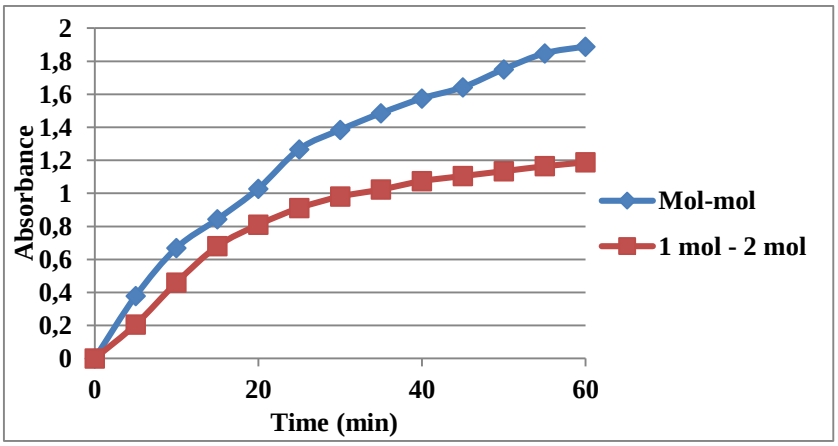


Figure 5: Oxidation of catechol in presence of **[L**$_1$/ Cu(CH$_3$COO)$_2$] (1/2) in MeOH

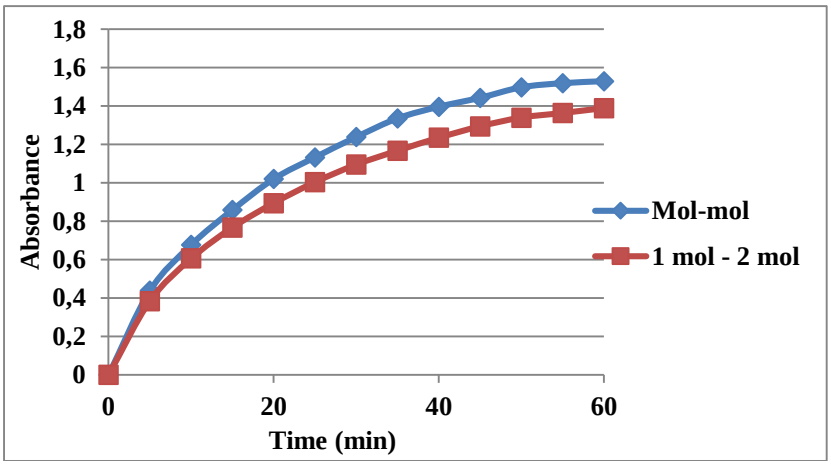


Figure 6: Oxidation of catechol in presence of **[L**$_2$/ Cu(CH$_3$COO)$_2$] (1/2) in MeOH

3.3. Effect of solvent

We chose three solvents to prove the effect of the media in such reactions (MeOH, THF and Acetonitrile) (Fig.7-8). As you can see from the Figures 7-8, the nature of solvent has a huge effect on the catechol oxidation rates. We can conclude that methanol is the good solvent for this reaction with the combination L$_1$/(Cu(CH$_3$COO)$_2$, Followed by THF then the acetonitrile. But for the combination L$_2$/(Cu(CH$_3$COO)$_2$, We have THF is better than MeOH and Acetonitrile, Du may be to the polarity of the solvents and the nature of the ligand.

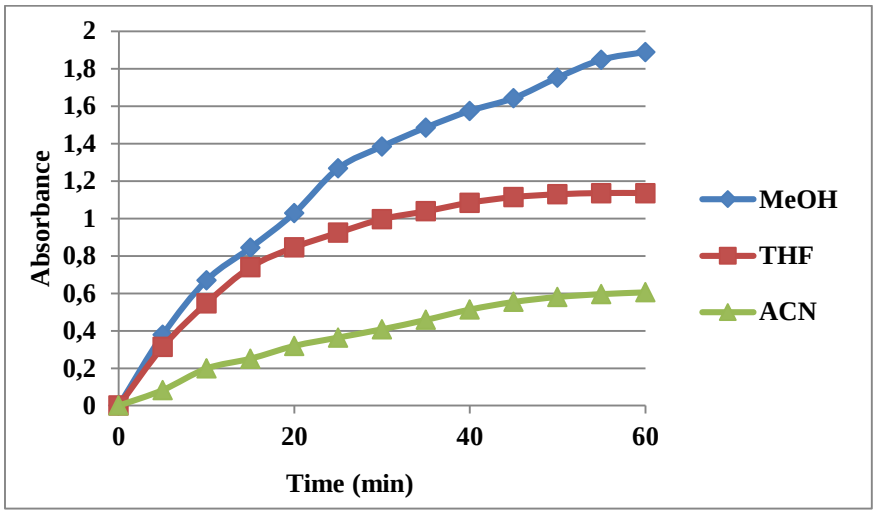


Figure 7: Catechol oxidation in presence of $L_1/Cu(CH_3COO)_2$ in different solvents

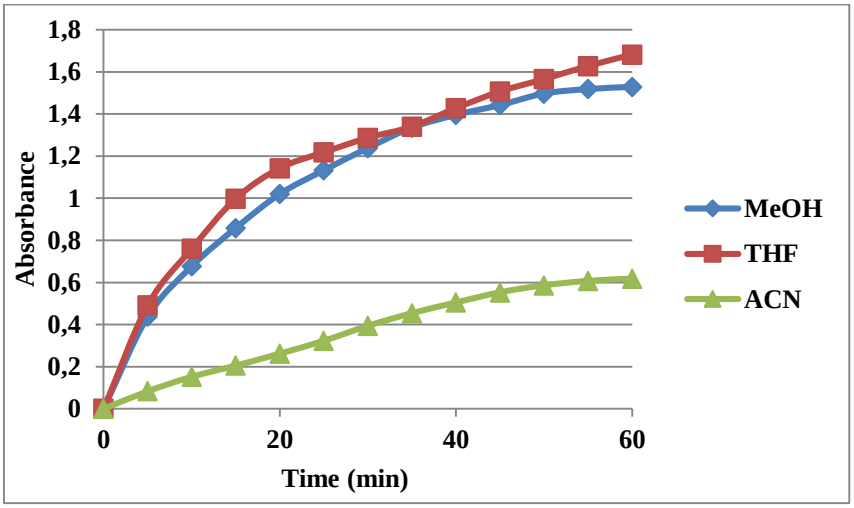


Figure 8: Catechol oxidation in presence of $L_2/Cu(CH_3COO)_2$ in different solvents

3.4. Kenitic studies

3.4.1. 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 acetonitrile, MeOH and THF solvents (Figures 9-11).

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. The parameters that we have determined are K_M and V_{max} [32]. The Michaelis kinetic parameter K_M represents the dissociation constant of the intermediate compound *catechol-Cat* (Figures 12-14). 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 2.

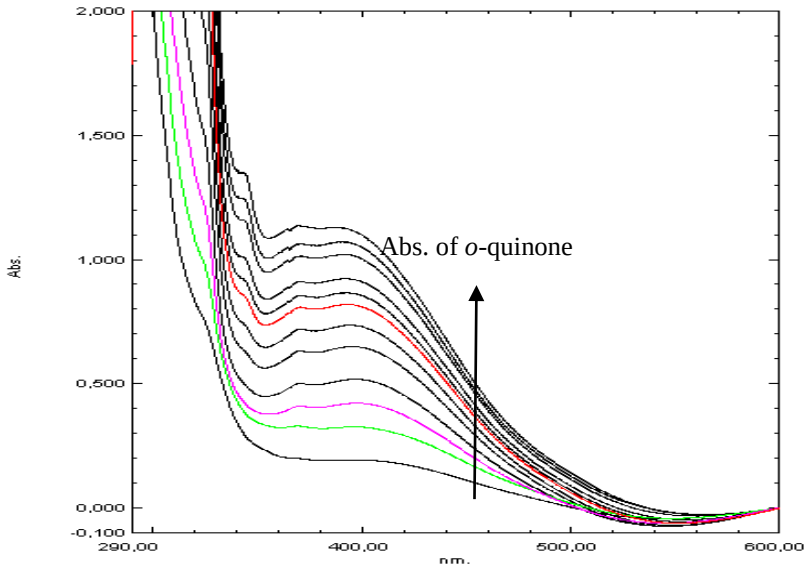


Figure 9: Absorbance of the *o*-quinone using $L_1/Cu(CH_3COO)_2$ in acetonitrile (ACN)

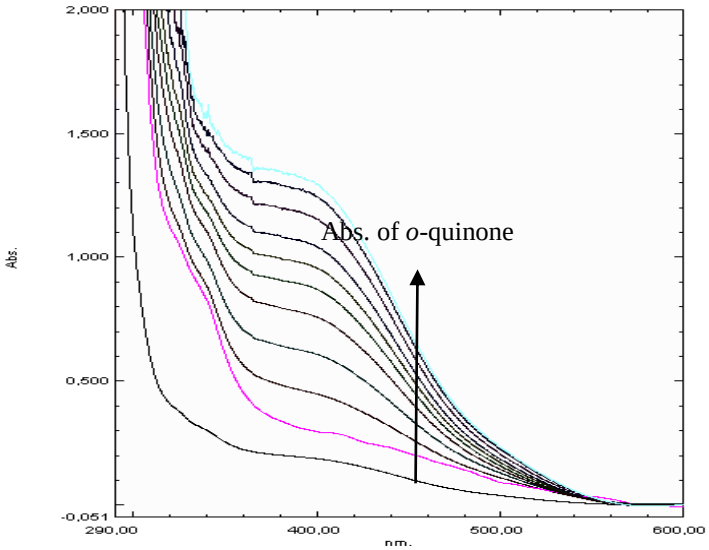


Figure 10: Absorbance of the *o*-quinone using $L_1/Cu(CH_3COO)_2$ in THF

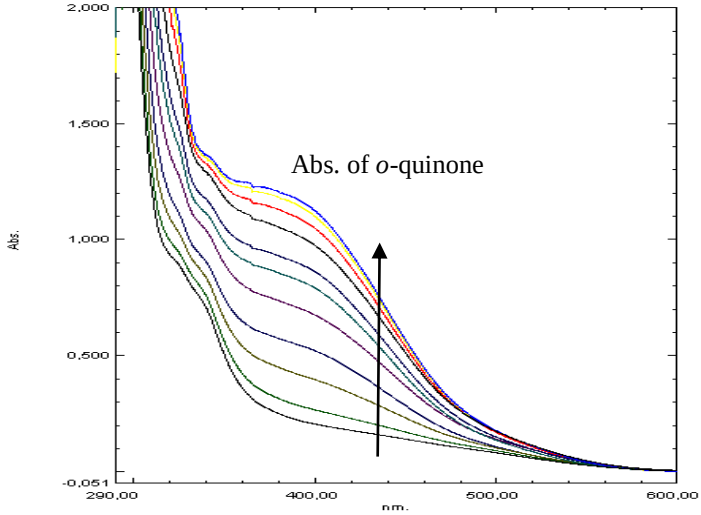


Figure 11: Absorbance of the *o*-quinone using $L_1/Cu(CH_3COO)_2$ in MeOH

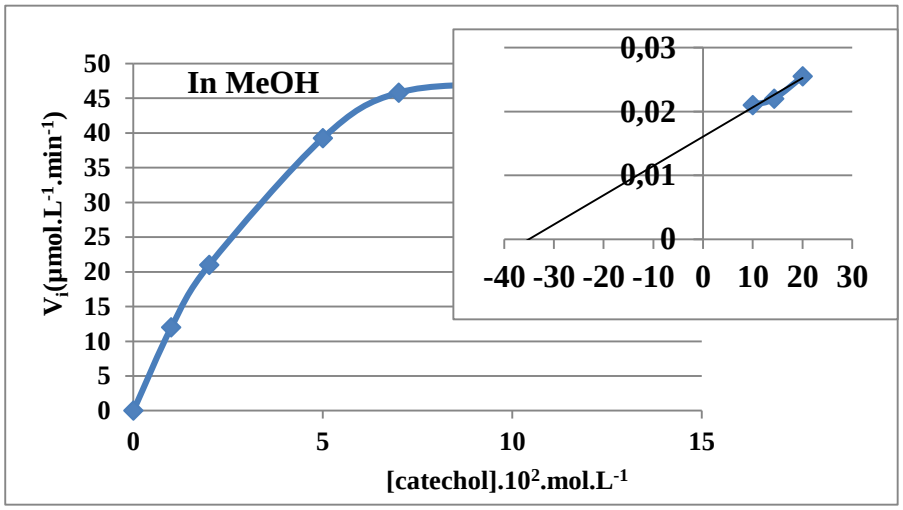


Figure 12: Reaction dependence on the concentration of catechol using L_1/[$Cu(CH_3COO)_2$] in MeOH

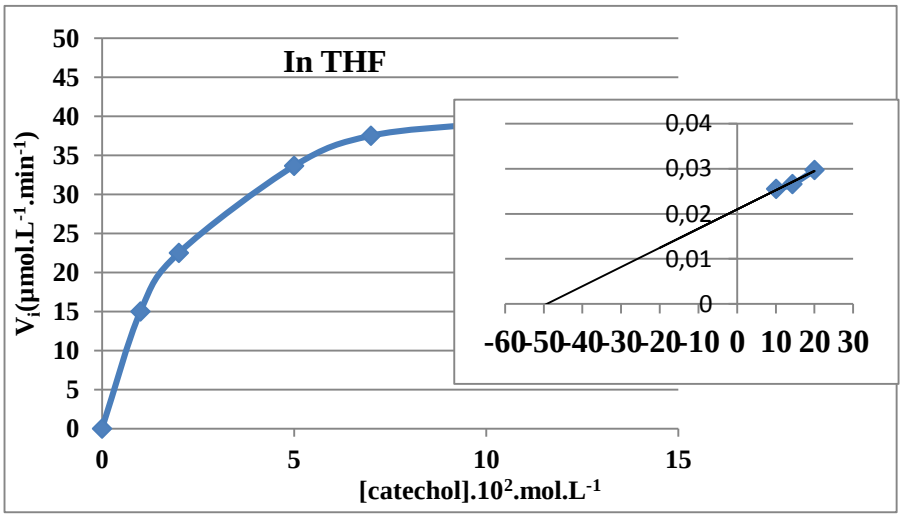


Figure 13: Reaction dependence on the concentration of catechol using L_1/[$Cu(CH_3COO)_2$] in THF

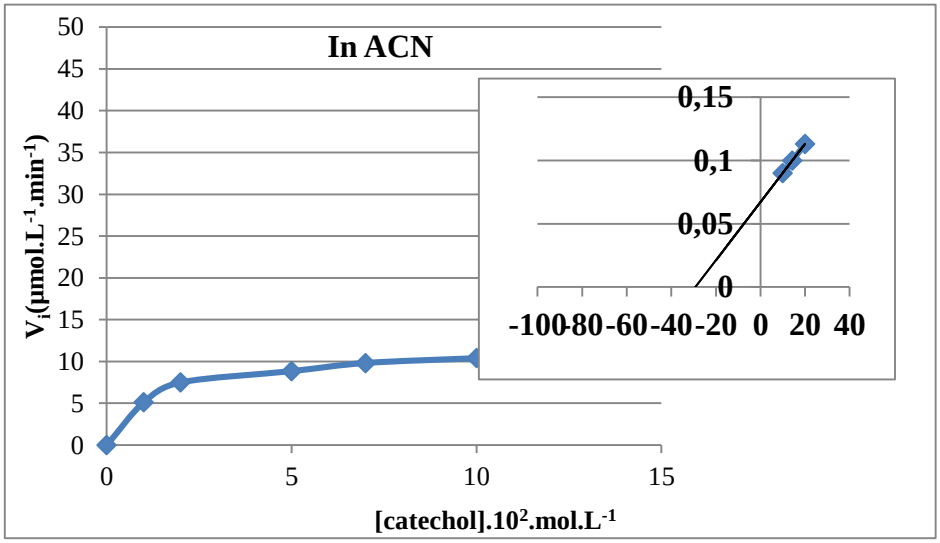


Figure 14: Reaction dependence on the concentration of catechol using L_1/[$Cu(CH_3COO)_2$] in ACN

Table 2: Kenitic parameters of the oxidation of catechol using $L_1/Cu(CH_3COO)_2$ in THF, MeOH and ACN

	THF	**MeOH**	**ACN**
V_m (µmol.L^{-1}.min^{-1})	39.13	47.25	10.4
K_M (mol.L^{-1})	2.10^{-2}	$2.86.10^{-2}$	$3.57.10^{-2}$

Conclusion

Two ligands L_1-L_2, were reported and examined for their catecholase activities at ambient conditions**.** The metallic complexes formed *in-situ* from L_1-L_2 and $Cu(CH_3COO)_2$, $CuSO_4$, $Cu(NO_3)_2$, $CuCl_2$, $CoCl_2$, $NiCl_2$ and $ZnCl_2$ show significant catalytic influence pathway on the oxidation of catechol to the *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, and the effect of the solvent are studied. The reaction follows Michaelis–Menten enzymatic reaction kinetics to determinate the kinetic parameters.

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