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Biomimetic catecholase studies: using *in-situ* prepared complexes by 1,2,4-triazole schiff bases and different metal salts

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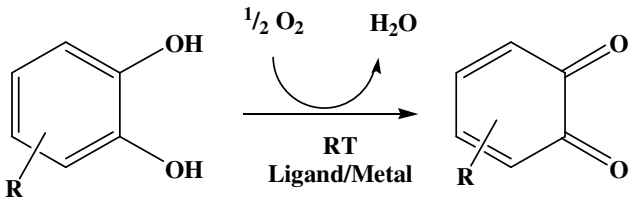
Abstract
In-situ metal complexes of some 1,2,4-triazole Schiff base ligands (**L_1 – L_{11}**) were examined for their catalytic activities. The dioxygen complexes of metals were generated by stirring metal salts and the 1,2,4-triazoles Schiff base ligands, it has been found that the oxidation of catechol is very efficient to give the appropriate *o*-quinone. We have found that ligand structure, solvent, concentration and substrate have proven huge influence on the combinations metal/ligand reactivities. The highest rate activity is given by the *in-situ* complex formed by ligand **L_{10}** and **$Cu(NO_3)_2$** which is equal to 11.52 µmol.$L^{-1}$$min^{-1}$. Also, the solvent has huge influence on the kinetic oxidation reaction system.

Keywords: triazole; catecholase; Schiff base compounds; metal salts; oxidation reaction.

1. Introduction
Complexes coordinated to multidentate heterocyclic ligands have found extensive use as models biomimitic proteins such as hemocyanin and tyrosinase [1]. These proteins are classified as Type 3 copper proteins because their dinuclear clusters [2-5]. Therefore, a notable advance in the understanding of the properties of these proteins has been achieved through the comparison of synthetic models to the naturally occurring molecules [6].



catechol
R = H; ^{t}-Bu; NO_2

***o*-quinone**
390- 400 nm

Ligands : **L_1-L_{11}**
Metals : $Cu(CH_3CO_2)_2$; $Cu(NO_3)_2$; $ErCl_3$; $NiCl_2$; $CoCl_2$; $ZnCl_2$.

Figure 1: Reaction model for catecholase studies

For this purpose several catechol derivatives were used in the literature (**Figure 1**). The catechol is a common substrate in catecholase enzyme research. 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 centre [7]. In the interest of finding a new *in-situ* complexe for such reaction, and also to understand the reactivity of such enzymes containing different metals. Herein, we report the influence of the diversity of eleven 1,2,4-triazole ligands combined with six different metal salts. The effect of different parameters towards oxidation of catechol derivatives to *o*-quinone correspondents will be discussed.

2. Experimental section

2.1. General

1,2,4-triazoles are class of nitrogen heterocyclic compounds which attract considerable attention in organic chemistry owing to their pharmacological and medicinal properties [8-10]. The 1,2,4- triazole Schiff base ligands **L_1 – L_{11}** (**Figure 2**) were known products [11].

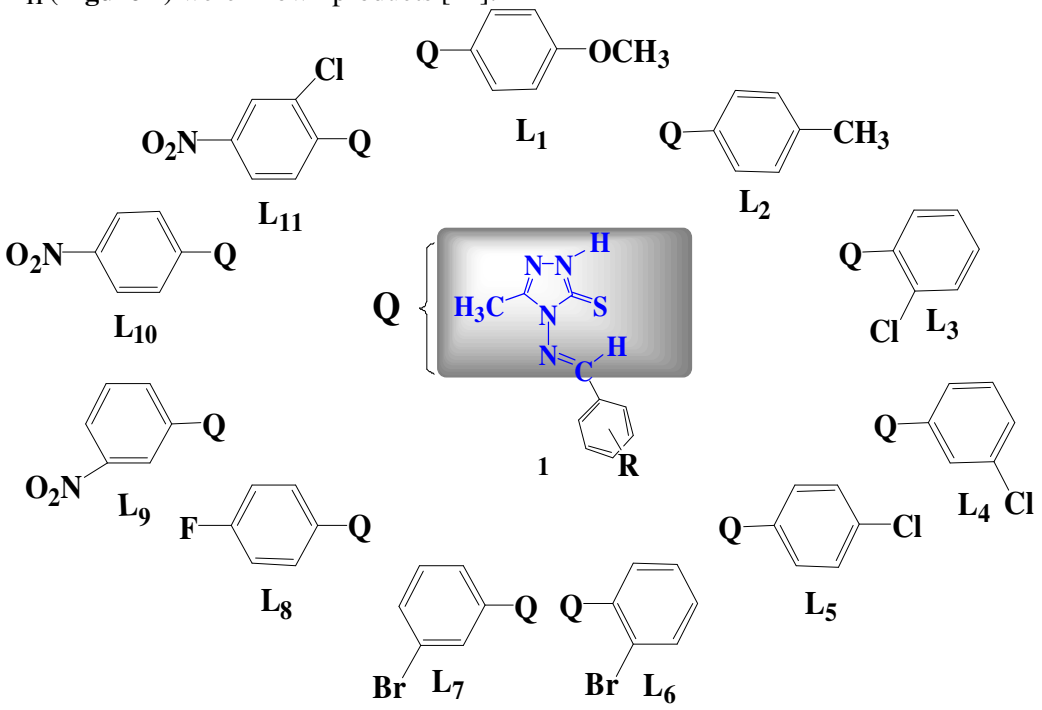


Figure 2: Structures des Ligands **L_1-L_{11}**

2.2. Catecholase Activity Measurements

Kinetic measurements were made spectrophotometrically on UV-Visible Cecil CE 292 Digital spectrophotometer, following the appearance of 3,5-di-tert-butylquinone over time at 25°C (390-400 nm absorbance maximum, ε = 1600 M^{-1} cm^{-1} in methanol; acetonitrile or in CH_2Cl_2, and ε = 1900 M^{-1} cm^{-1} in THF). The metal complex (prepared *in-situ* from copper salt and the ligand, 0.3 mL of 10^{-3} M in methanol solution) [12-23] 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. Results and discussion

Herein we report the catecholase studies using some Schiff bases derived from 4-amino-5-methyl-1,2,4-triazol-3-thione and the study of their biomimetic properties toward this reaction (**Figure 2**). The progress of the catechol oxidation reaction is conveniently followed monitiring the strong absorbance peak of *o*-quinone in the UV/Vis spectrophotometer. The metal complex (prepared *in-situ* from metal salts and the ligand [12-23] and a solution of catechol derivatives were added together in the spectrophotometric cell at 25°C. Formation of the corresponding *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 of the reaction for different metal complexes and ligands. As can be seen from **Table 1**, all of the complexes prepared *in situ* with this kind

of ligands catalyze the oxidation reaction of catechol to *o*-quinone with the rate varying from a high of **11.52** µmol.L^{-1}.min^{-1} for the **L_{10}/Cu$(NO_3)_2$** complex to a low of 0.03 µmol.L^{-1}.min^{-1} for **L_6/$ZnCl_2$.** These rates are comparable to the values reported by Mouadili et *al.,* [23]. The catalytic activities depend strongly on both the R substituents of the ligand and the type of inorganic anion.

Table 1: Oxidation rate of catechol in THF by 1,2,4-triazol Schiff base ligands (**L_1-L_{11}**) and metal complexes [Activity (µmol. L^{-1}. min^{-1})]

L/M	$Cu(CH_3COO)_2$	$Cu(NO_3)_2$	$ErCl_3$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
L_1	3.95	2.02	0.93	0.31	2.48	0.21
L_2	1.65	0.92	1.42	1.96	4.11	0.91
L_3	2.07	2.81	2.76	0.83	2.61	0.26
L_4	2.02	2.13	2.44	0.64	3.68	0.72
L_5	2.01	2.18	3.21	0.74	3.46	0.76
L_6	1.58	2.26	3.33	0.03	3.28	0.03
L_7	1.93	1.98	2.80	0.45	3.52	0.46
L_8	1.91	2.94	1.12	0.05	1.99	0.10
L_9	2.31	2.10	0.87	0.16	1.79	0.12
L_{10}	2.28	**11.52**	2.20	0.94	2.20	0.09
L_{11}	1.92	2.06	3.92	0.13	5.71	0.19

3.1. Effect of ligand concentrations

To understand the effect of ligand concentration to form the catalyst of the oxidation reaction of catechol to *o*-quinone, we realized this reaction using different concentration of ligand and metal ion (L / M: 1 / 1; 2/1; 1/2), after monitoring the absorbance of *o*-quinone for each proportion, we found the spectra of **Figure 3.**

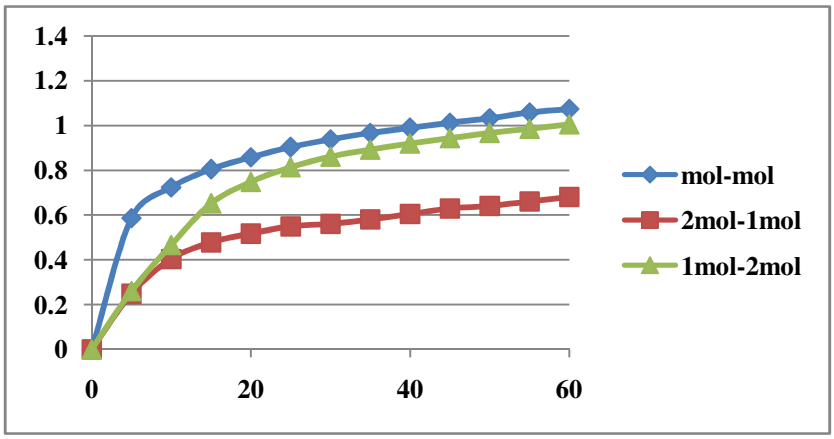


Figure 3: Oxidation of catechol rates in presence of **L_{10}/ Cu$(NO_3)_2$** with different concentrations

The results obtained show the change in absorbance at 390 nm as a function of time during the first hour of the reaction, which explains the combinations used as catalyst catalyze the reaction well studied, and it is clear that there to differences in the absorbance values for each combination, according to these values, the combination formed by a mole-mole / metal-ligand appears the best catalytic condition for this reaction. This allowed us to consider the influence coordination environment the catalytic activity of the combinations such as: (a) the geometry imposed by the ligand on the metal ion (structure of the complex). (b) The characteristics of the steric effect of ligand.

3.2. Effect of solvent

In this part, we realized the oxidation reaction catalyzed by the combination [L_{10}/$Cu(NO_3)_2$] in different solvent (THF, MeOH, ACN, DCM) after monitoring the evolution of the absorbance product of the reaction, we found the following spectra (**Figure 4**).

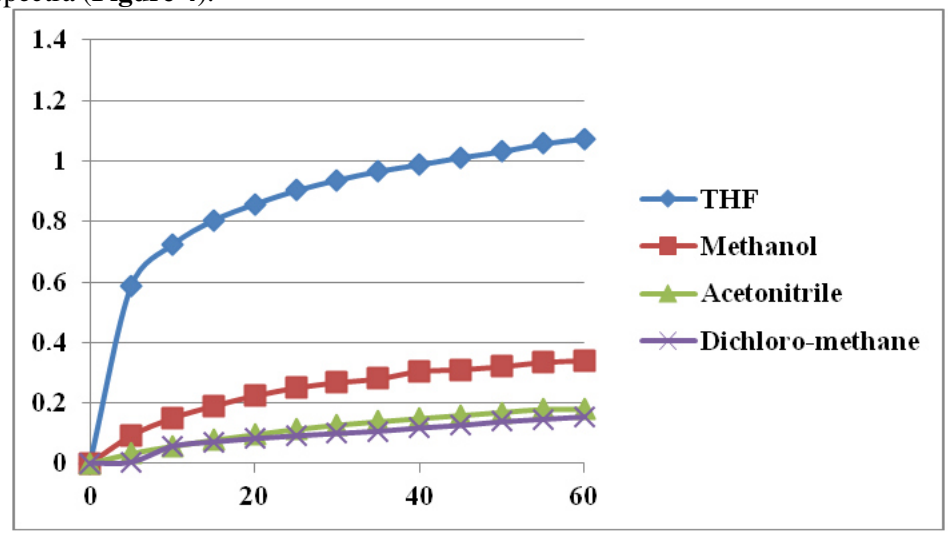


Figure 4: Oxidation of catechol rates in presence of L_{10}/ $Cu(NO_3)_2$ in different solvents

As you can see from the **Figure 4**, the spectrophotometric monitoring, two fortunes of the catechol oxidation catalyzed reaction [L_{10}/$Cu(NO_3)_2$] in different solvents. The catechol concentration is about 10^{-1} mol.L^{-1}. The intensity of the absorbance of *o*-quinone increases progressively over time. From the spectra in **Figures 4**, it is clear that the catalytic conversion of catechol to *o*-quinone is influenced by the nature of the solvent. According to studies by Banu et *al.,* [23] in 2012, the special properties of solvents, such as dielectric constant, dipole moment and polarity complex in these solvents have no effect on the catalytic conversion of substrate corresponding *o*-quinone. So we can consider that may be the coordination solvent power is the factor that directs the catalytic activity of the combination [L_{10}/$Cu(NO_3)_2$]. Our argument on this point is that during catalysis there is a competition between the solvent molecules and the substrate (catechol) for interacting with the metal center. It is thus obvious that the more solvent coordinating power, the greater the solvated catalyst more stable, and therefore the probability of substrate interaction with the metal center is lower. We found that THF has the highest catalytic activity of our combination [L_{10}/$Cu(NO_3)_2$], followed by MeOH, ACN, and finally DCM. So for this study, the coordination solvent power is the key factor in the direction of the catalytic efficiency of the catalysts.

*3.3. Effect of substrat*e

To discover the substrate effect on the catalytic activity of our combinations [L_{10}/$Cu(NO_3)_2$], the kinetics of the oxidation reaction of different substrates (catechol, 3,5-DTBC and nitro-catechol) was studied by observing the evolution of absorbance versus time at a wavelength of 390 nm for the product of oxidation of catechol and 400 nm for the products of the oxidation of two other substrates in THF. according to **Figures 5-6**, a difference of the absorbance value for each substrate which shows that the substrate influence far catecholase activity of the systems studied and 3,5-DTBC can be easily oxidized to the corresponding quinone is observed so it remains the best substrate for reproducing the catalytic activity of the enzyme catecholase, because of its low potential for the couple quinone catechol, leading to easy oxidation to quinone correspondent and also because of its bulky substituent which limits the degradation of the substrate, this was confirmed in 2013 by Ronan and colleagues [24].

3.4. Kinetic study:

The catalytic activity of a complex can be evaluated by determining the kinetic parameters of the oxidation reaction. In most of the studies described in the literature [24-25], the rate constants are determined by applying the model of Michaelis-Menten commonly used in enzymatic catalysis. This model is based on the hypothesis of a catalytic mechanism consisting in fixing the substrate (cat, DTBC, ...) on the enzyme (complex) with the formation of a substrate-enzyme intermediate and product formation (*o*-quinone, DTBQ, ...) and salting out of

the enzyme. Under these conditions, the constants of pseudo-first order are determined with respect to the substrate or the enzyme according to the default reagent in the solution. In practice, this amounts to performing manipulations on solutions with varying the substrate concentration by maintaining the catalyst concentration constant, and vice versa.

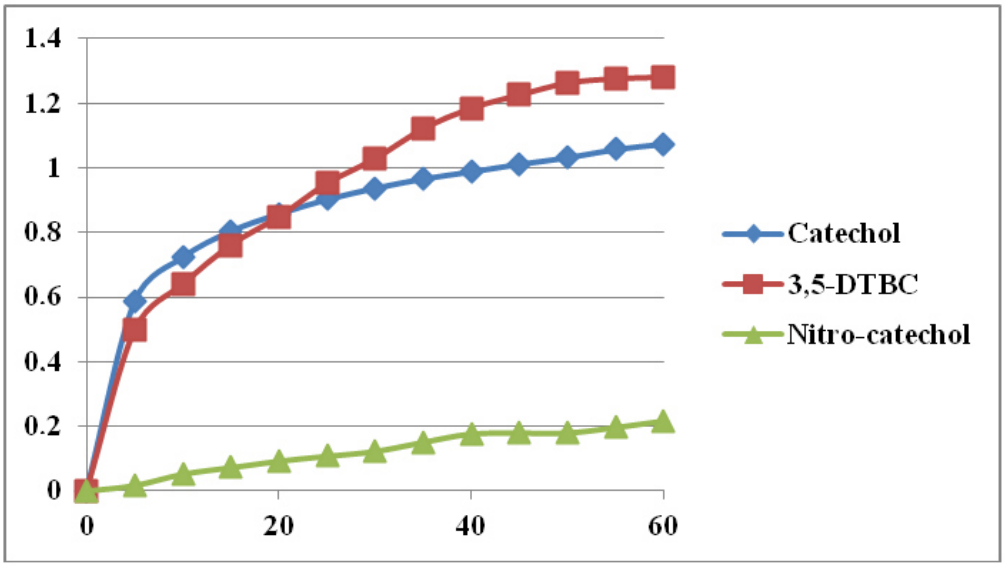


Figure 5: Oxidation rates of three substrates using [$\mathbf{L_{10}/Cu(NO_3)_2}$] in THF

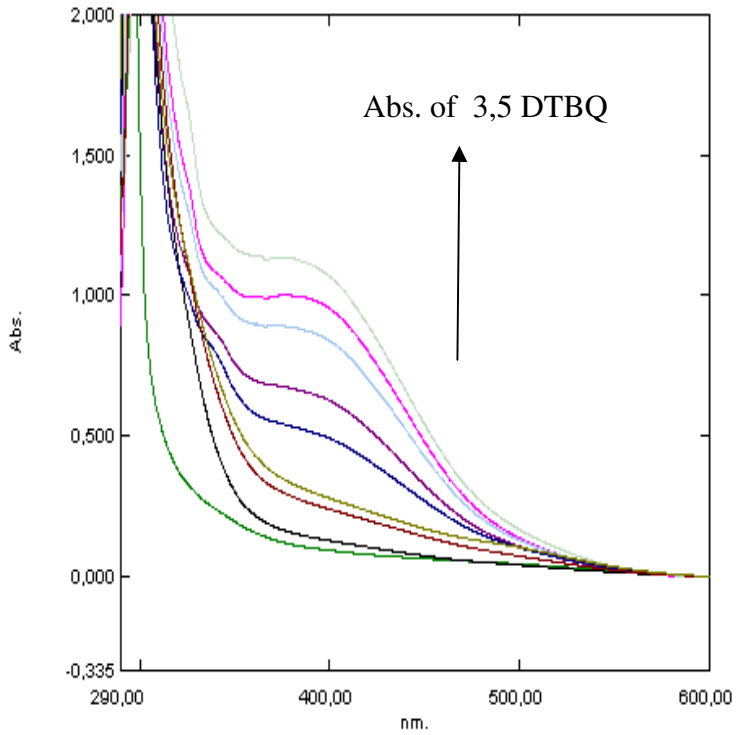


Figure 6: Absorbance spectra evolution of (3,5-DTBQ) using [$\mathbf{L_{10}/ Cu(NO_3)_2}$] in THF

We determined the speed V of initial reaction, considering the changes in absorbance at 400 nm during the first five minutes. The operations are performed on different solvents (THF, MeOH, ACN, and DCM): the catalyst concentration is 2.10^{-3} mol L^{-1} and that of catechol varies from 10^{-2} to 4.10^{-1} mol.L^{-1}. The results are shown on **Figures 7-10**.

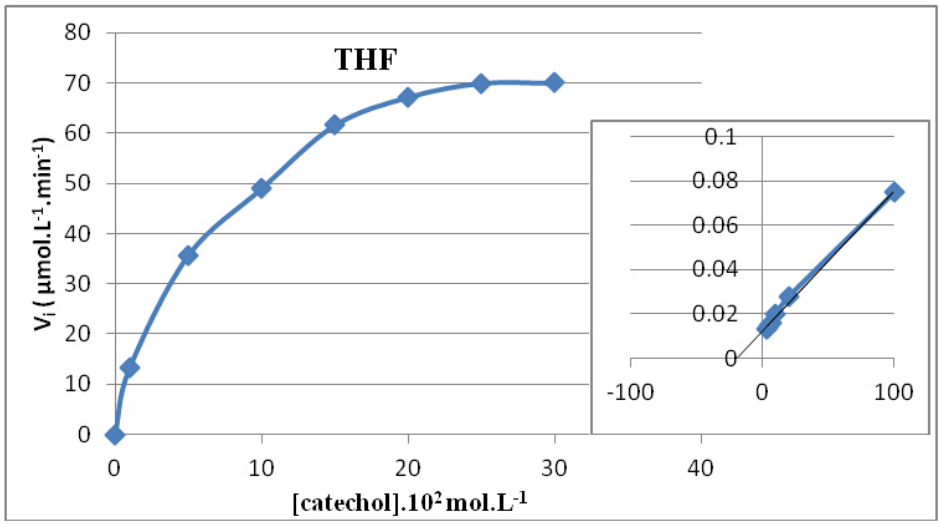


Figure 7: Reaction dependence on the concentration of catechol using **L_{10}/[$Cu(NO_3)_2$]** in THF

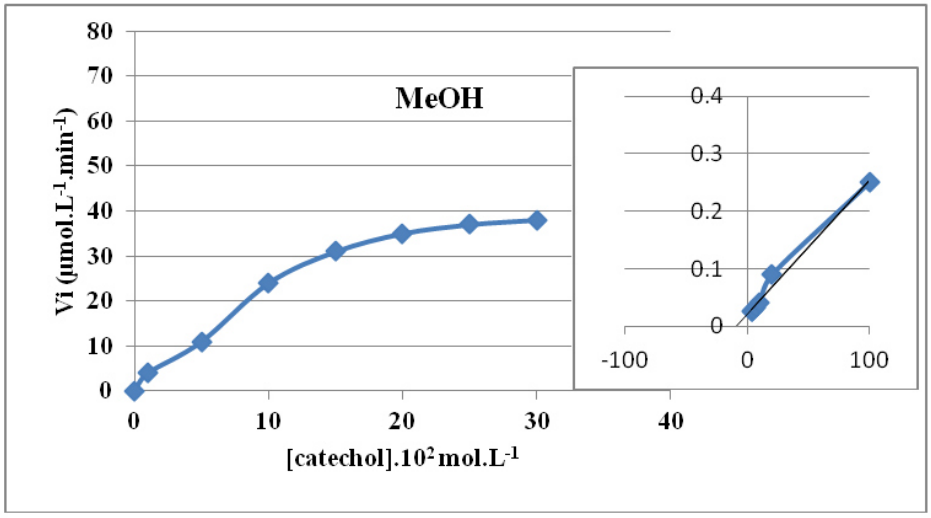


Figure 8: Reaction dependence on the concentration of catechol using **L_{10}/[$Cu(NO_3)_2$]** in MeOH

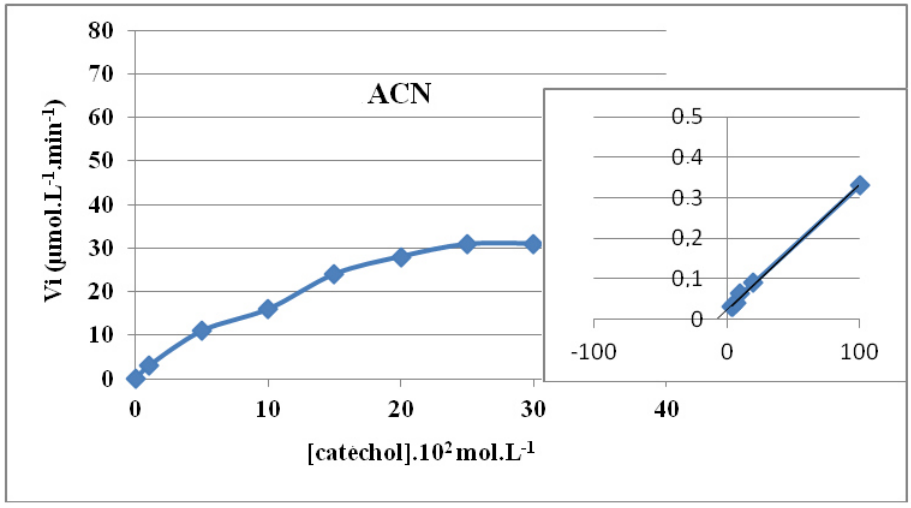


Figure 9: Reaction dependence on the concentration of catechol using **L_{10}/[$Cu(NO_3)_2$]** in Acetonitrile

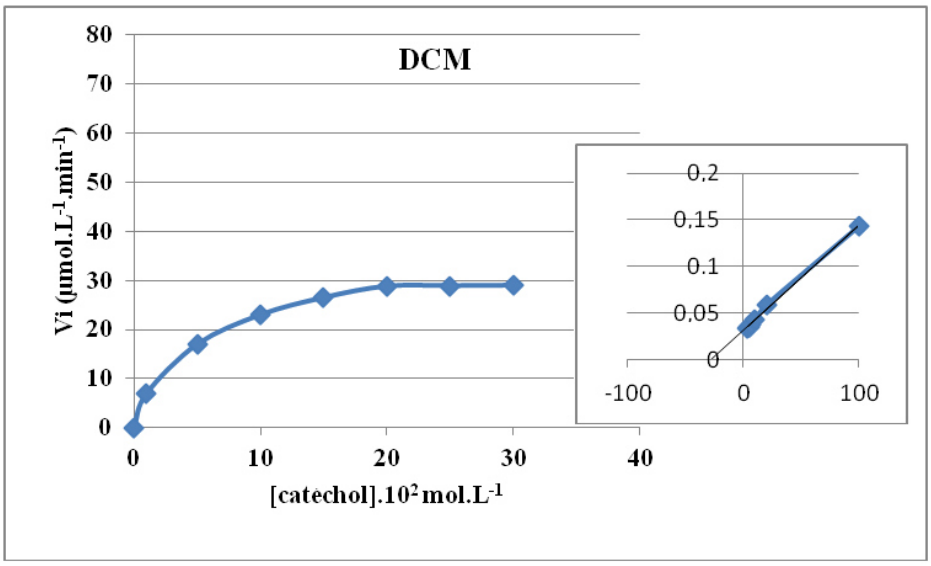


Figure 10: Reaction dependence on the concentration of catechol using $\mathbf{L_{10}/[Cu(NO_3)_2]}$ in CH_2Cl_2

Table 2 : Kinetic parameters of catechol oxidation using $\mathbf{L_{10}/Cu(NO_3)_2}$ in Different solvents

	THF	**MeOH**	**Acetonitrile**	**CH_2Cl_2**
V_m (µmol.L^{-1}.min^{-1})	70.01	37.02	30.87	28.91
K_M (mol.L^{-1})	0.05	0.1	0.13	0.037

For low concentrations of catechol, the reaction rate significantly increases and decreases from 100 equivalents (2.10^{-1} mol L^{-1}): The reaction is probably stoped by forming other products after adding an excessive amount of substrate. A test was conducted to 300 equivalents of catechol (substrate) and we found that the reaction rate was virtually zero. We can explain this by the probable damage to the in-situ formed complex upon addition of catechol, was observed in the case of complex studied by Mukherjee et *al.,* [26] considering competition between the bridge connecting the metal cations and the bridge catecholate when the concentration of DTBC increases. On the other hand, **Figures 7-10** and **Table 2** show that the values of K_M is different with the change of solvent, they range from 0.05 mol L^{-1} for THF which are the best solvent for catalytic study of this series in catecholase oxidation and 0.13 mol L^{-1} for acetonitrile which appears not suitable solvent for this catalytic study

Conclusion

In this study we focused our efforts on the study of the effect of eleven triazole schiff bases on the catecholase activity of *in-situ* prepared complexes. The results obtained show that the nature and concentration of ligands and metallic salt], the nature of solvent and concentration of the catechol substrate have a great effect on the studied combinations in catecholase activity.

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