Catecholase Activity Investigations using *in situ* Copper Complexes Continuing Schiff Base Derivatives with a Theoretical Calculation

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ABSTRACT

The study of catecholase activity of a series of Schiff base compounds using in situ copper complexes of 4-hydroxy-6-methyl-3-(1-(phenylimino)ethyl)-2H-pyran-2-one derivatives has been reported. The reaction rate depends on four parameters: The nature of the substitution in para position to the benzene ring, the nature of counter anion, the concentration of ligand and the nature of solvent. The highest rate activity is given by complex resulting from one equivalent of ligand **L2** and two equivalents of copper acetate in methanol, which equal to 62.25 µmol.min^{-1}.L^{-1}.In other part, a theoretical study of such ligands using the semi-empirical method AM1 were also investigated. A good relationship founded between the maximal reaction rate (V_{max}) and the HOMO energy (Pearson correlation: r=-0.794).

Keywords : Schiff base; Catecholase; copper complexes; catalyst; semi-empirical method; AM1.

INTRODUCTION

Transition metal ions have an important role in biochemistry and biomimetic systems and may provide the basis of models for active sites of biological targets[1]. The presence of copper (II), iron (II) and zinc (II) is crucial in many biological processes. These metals are present in metallo-

enzymes that involve the coordination of a metal ion as an active site and in which the metal plays the role of catalyst[2]. They also participate in the electron transport of O_2, and the degradation of the superoxide ion O_2^- [3-5]. The coordination chemistry specially of dinuclear Cu(II) complexes has been developed very well with the goal of miming the active sites of the type 3 copper proteins/enzymes such as hemocyanin, tyrosinase and catechol oxidase. The oxidation of a wide range of *o*-diphenols to *o*-quinones is catalyzed by the catechol oxidases[6]. Thus, to understand more about this biological process and to search for new ligands that are able to contribute with other metals miming bio-organism puzzles, we examined a series of Schiff base compounds . These compounds have a wide range of applications, for example, catalyst, nonlinear optical materials, electroluminescent materials and others[7]. The synthesis of novel Schiff bases and the research of their physiological activities have become important due to their biological activities[8-10]. In continuation of our biometric work[11], we focused on the study of catecholase activity by using these ligands with different transition metals especially the copper via the oxidation of catechol to *o*-quinone.

EXPERIMENTAL

Chemistry of the studied ligands

All reagents were purchased from commercial sources and used without further purification. The ligands were prepared, and characterized according to literature[12], via an efficient condensation of an amine and dehydroacetic acid **L0** in ethanol. The structures are shown in Scheme 1.

Method of catalytic measurements

The measurements were made spectrophotometrically on UV–Vis spectrophotometer (in the COSTE: Centre de l'Oriental des Sciences et Technologies de l'Eau), following the appearance of *o*-quinone over time at 25 °C (390 nm absorbance maximum**,** µ = 1,600 $L.mol^{-1}.cm^{-1}$ in methanol[13]). The complexes were prepared *in situ* by successively mixing 0.30 mL of a solution (2×10^{-3} M) of CuX_2, nH_2O (X = Cl⁻, Br⁻, NO_3^-, CH_3COO^- or SO_4^{2-}) with 0.15 mL of a solution (2×10^{-3} M) of ligand, then adding 2 mL of a solution

of catechol at a concentration of 10^{-1} M. After that, the evolution of product absorbance was followed at 390 nm according to time after regulation in zero[11] (Scheme 2).

RESULTS AND DISCUSSION

Catalytic activity studies

After realization of catechol oxidation reactions catalyzed by copper complexes with ligands **L0**–**L7**, the evolution of *o*-quinone absorbance was followed at 390 nm on the UV spectrometer UV–vis, we note that the catalytic activity varies from a complex to the other one, the obtained results for $Cu(CH_3COO)_2$ salt are summarized in below (Fig. 1)(more figures about the absorbance of o-quinone with different metal salts in the supplementary material) and the V_{max} rates of the catechol oxidation were calculated in a time range of 5 min where the slope of the absorbance graph is maximal and collected in Table 1. In this part, we take one equivalent of ligand for two equivalents of metallic salt. In addition, the results obtained with **L0** is not recorded here because it is too weak as if we were used the catechol alone.

Table 1: Maximum rates of catechol oxidation in methanol

Ligands	V_{max} (µmol.min⁻¹.L⁻¹)
L0	-
L1	43.75
L2	62.25
L3	34.12
L4	40.25
L5	38.50
L6	3.62
L7	23.62

Table 2. Oxidation rate of catechol oxidation in methanol

Ligands	V_{max} (µmol.min⁻¹.L⁻¹)
L2	62.25
L7	23.62
L8	24.07
L9	5.86

According to the obtained results, we notice that all copper complexes, formed *in situ* from ligands **L1**–**L7** and copper salts ($Cu(CH_3COO)_2$,$CuBr_2$,$CuCl_2$, $Cu(NO_3)_2$,$CuSO_4$) , catalyze the oxidation reaction of catechol to *o*-quinone, but with different rates which varying from 0.18 µmol.min^{-1}.L^{-1} for the complex formed from ligand **L6** and the metallic salt $CuSO_4$ (weak catalyst) to 62.25 µmol.min^{-1}.L^{-1} for the complex formed from ligand **L2** and metallic salt $Cu(CH_3COO)_2$ (best catalyst). At this step, these results can be explained by the intervention of two factors: firstly, the nature of the core group on the para position of the benzene ring, in fact and in general , the donor group can increase the complexing properties of the ligand by the enrichment of the benzene ring, and promote stability in the complex. However, in presence of an electron attractor group, a disadvantage complex formation occurs by reducing the electron density on the sites of chelation and, therefore, reducing the rate of correspondence between the substrate and the complex. Secondly, the nature of counter-ion, so if it is strongly bonded to the metal as in the case of NO_3^-, Cl$^-$ and Br$^-$ ions, the binding of the substrate on the metal will be difûcult, therefore reducing the catalytic power of the corresponding complex. Otherwise, the bonds formed between the metal and its counter-ion are not as strong (case of the CH_3COO^- ion), so the substrate can be replaced easily, which positively affects the rate of the reaction. In this part, we use different parameters to study the catalytic activity of the product **L2** which gave the best results.

Effect of ligand concentration on the catecholase activity

In this study, the evolution of the oxidation

of catechol to the *o*-quinone it's followed in the presence of variable equivalents of ligand **L2** and the metallic salt $Cu(CH_3COO)_2$. Three different

Table 3: Oxidation rate of catechol oxidation in presence of the ligand and the copper metallic salt $Cu(CH_3COO)_2$

Ligands	V_{max} (µmol.min^{-1}.L^{-1})
L1	43.75
L2	62.25
L3	34.12
L4	40.25
L5	38.50
L6	3.62
L7	23.62
L8	24.07
L9	5.86

Table 4: Molecular orbitals energies of ligands L0-L9 calculated with AM1

Ligands	Energy (eV)		
	E_{HOMO}	E_{LUMO}	$\Delta E = E_{LUMO} - E_{HOMO}$
L0	-10.040	-0.574	9.465
L1	-8.539	-0.756	7.783
L2	-8.791	-0.736	8.054
L3	-8.818	-0.829	7.988
L4	-8.612	-0.721	7.890
L5	-8.413	-0.715	7.698
L6	-9.161	-0.935	8.226
L7	-8.080	-0.665	7.415
L8	-8.349	-0.717	7.632
L9	-8.095	-0.714	7.380

Table 5: Correlation between V_{max} and E_{HOMO}

Correlations		HOMO energy	V_{max}
HOMO energy	Pearson Correlation	1	-0.794[*]
	Sig. (2-tailed)		0.019
	N	8	8
V_{max}	Pearson Correlation	-0.794[*]	1
	Sig. (2-tailed)	0.019	
	N	8	8

[*]. Correlation is significant at the 0.05 level (2-tailed).
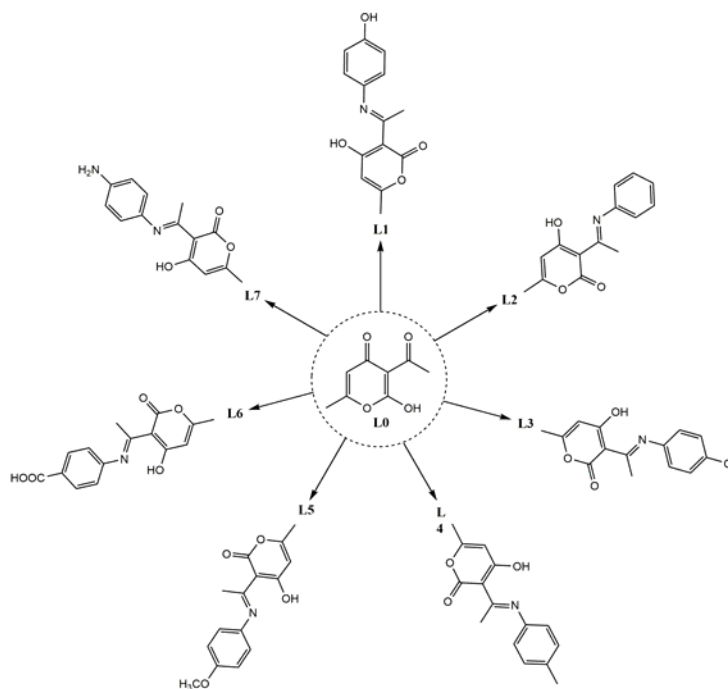
Scheme 1: Chemical structure of ligands
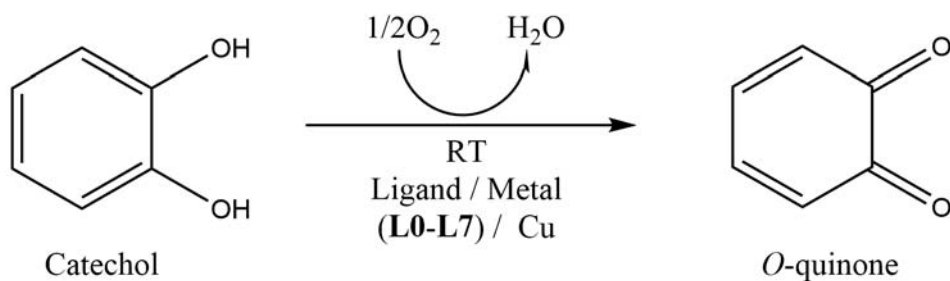
Scheme 2: The oxidation model reaction of the catechol to *o*-quinone
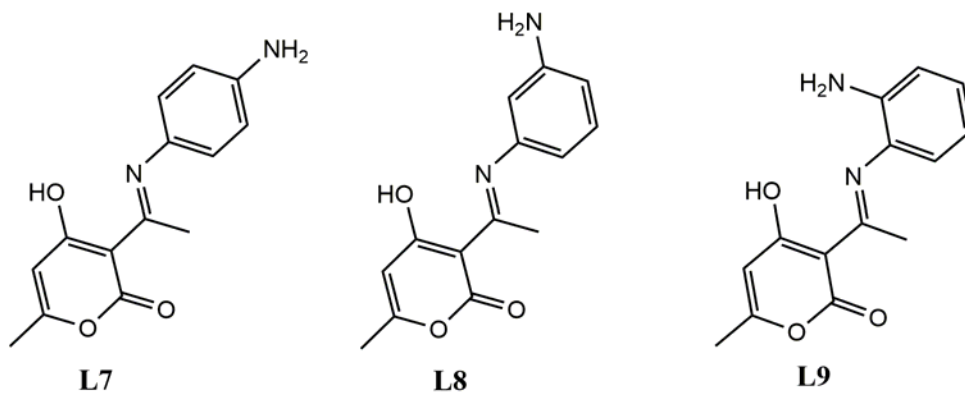
Scheme 3: Structure of used ligands

concentration are used: One equivalent of **L2** with one equivalent of Cu(CH$_3$COO)$_2$; Two equivalent of **L2** with one equivalent of Cu (CH$_3$COO)$_2$ and One equivalent of **L2** with two equivalent of Cu (CH$_3$COO)$_2$.

From the figure above, when the increasing of the concentration of the metal salt in first 35 min happens, the absorbance increase as will, after that, the absorbance decrease due to the precipitation of the a complex in the cuvette. Also
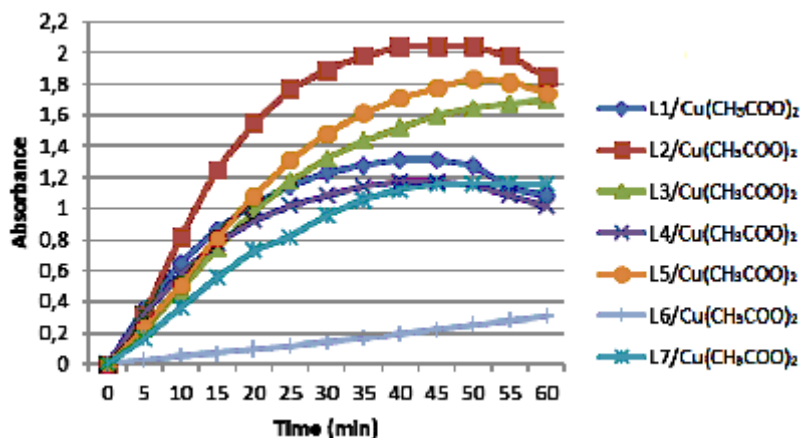

Fig. 1: Catechol Oxidation in the presence of different ligands L1-L7 and the metallic salt Cu(CH$_3$COO)$_2$ in methanol
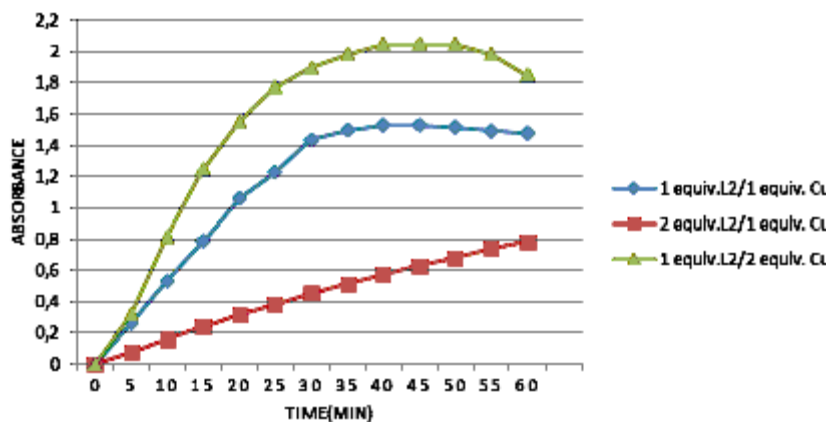

Fig. 2: Catechol Oxidation in the presence of variable equivalents of ligand L2 and the metallic salt Cu(CH$_3$COO)$_2$
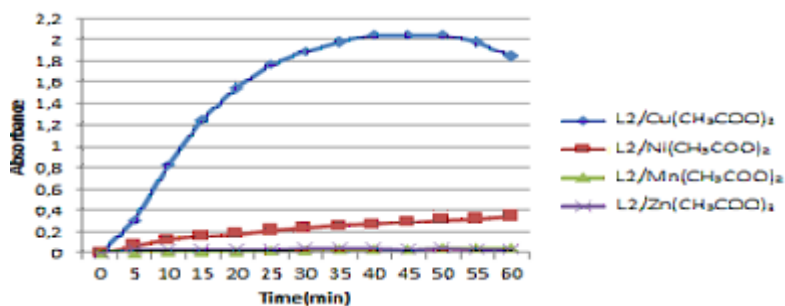

Fig.3. Catechol oxidation in the presence of metal complexes formed with L2 (1 equivalent of ligand for 2 equivalents of metallic salt)

the calculation of the oxidation rates, confirmed the absorbance values founded. From these observations, we can conclude, that the efficiency of our catalyst increase in presence of an excess of metal relative to the ligand, this result can be explained by the presence of two Cu(II) ions into the enzyme catecholase structure as site active, which granted firstly as an idea that our complex must contain two copper ion to in order to make its catalytic function happens in good way, and secondly as an idea about our model complexes which could be seen as functional or structural models for catechol oxidases or related copper containing enzymes.

Effect of the acetate salts

From the precedent results, what would be observed mainly is that the best results are showed
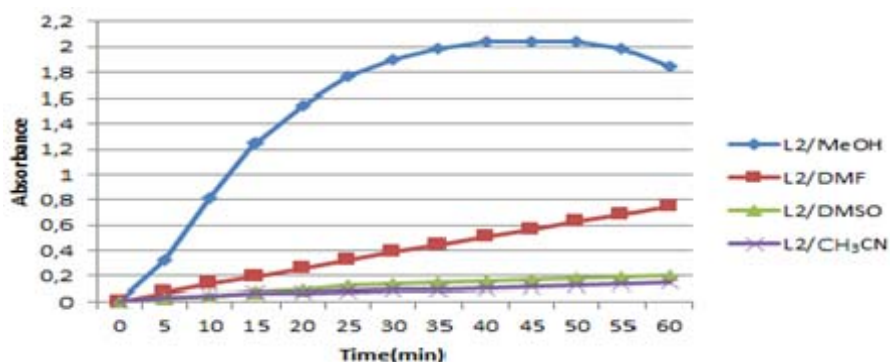

Fig. 4: Catechol oxidation in the presence of copper complexes formed with L2 in different solvents. (1 equivalent of ligand for 2 equivalents of metallic salt)
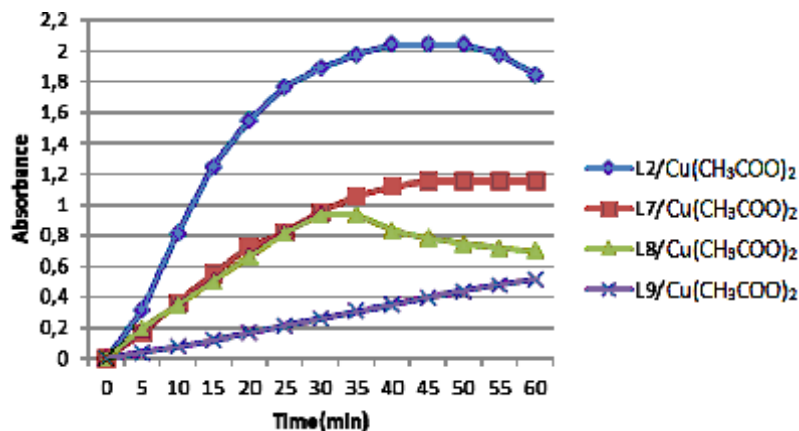

Fig. 5: Catechol oxidation in the presence of copper complexes formed with L7, L8 and L9. (1 equivalent of ligand for 2 equivalents of metallic salt)
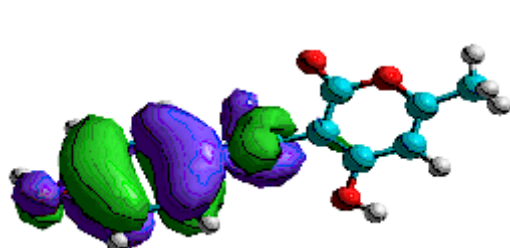

Fig. 6(a). The HOMO orbital of L1

Fig. 6(b). The LUMO orbital of L1

with Cu $(CH_3COO)_2$. Therefore, for identify if the CH_3COO^- ion have an effect on the catalytic activity, we were used in this study different acetate salts with different metal (Cu, Zn, Ni and Mn).

The obtained results, showed very clearly that Cu $(CH_3COO)_2$ give the best results with a oxidation rate equal to 62.25 µmol.min^{-1}.L^{-1}, followed by Ni $(CH_3COO)_2$ with a value of 5.93 µmol.min^{-1}.L^{-1}, when the other salts give a very low rate < 1 µmol.min^{-1}.L^{-1}. As we can see here, the acetates has no effect on the oxidation rate in these conditions, but the difference between the rate values it is due to the metal ion which is very logical, because the reduction potential of (Cu $^{2+}$)(E^0 =0.34V) is more higher or favorable than the other metals tested, which make it more reactive than (Ni^{2+})(E^0 =-0.25 V),(Zn^{2+})(E^0 =-0.76 V) and (Mn^{2+})(E^0 =-1.18 V).

Solvent effect

The effect of the solvent on the oxidation reaction with ligand **L2** and Cu $(CH_3COO)_2$ salt were studied in different solvents (MeOH, DMF, DMSO and CH_3CN). As a simple comparison, we observe that the absorbance in MeOH is higher than in other solvents with a maximal value of 2.044 after 45 min of experience, before decreasing after that due to the precipitation of a complex in the cuvette, which is not the case in other solvents specially the DMF because this complex is very soluble in this solvents. So, the methanol is better solvent than the others for this reaction, and that can be explained by its

protic and polar nature, because this kind of solvents are very known by their strong solvation of various anions X$^-$ (in our case X$^-$ = Cl$^-$, Br$^-$ NO$_3^-$, CH$_3$COO$^-$ and SO$_4^{2-}$) through hydrogen bonds, which make them isolated and therefore are not very reactive in this type of solvents. The copper cations are also solvated by the electronegative pole of the solvent but is weaker than the solvation by a hydrogen bond, and became moderately reactive.

On the other hand, we observe that the positive charge is diffused, whereas the negative charge is concentrated on a single atom in the dipolar and aprotic solvents (like DMF, DMSO,CH$_3$CN,…) which mean that kind of solvents are very strongly solvating the (Cu^{2+}) cations making them little or no reactive. For this, the formation of the copper complex is very difficult in this case, which negatively and indirectly affects the oxidation rate of the catechol to the *o*-quinone.

Substitution effect

The effect of substitution was studied for three ligands **L7**, **L8** and **L9** respectively substituted in Para, Meta and Ortho by a NH$_2$ group (Scheme 3), the Cu $(CH_3COO)_2$ is used as metal salt and the methanol as solvent.

Comparing the unsubstituted ligand **L2** with **L7**, **L8**, and **L9**, which have the same molecular formula, we note clearly that the existence of a substituted group on the benzene ring play an important role in the decrease of the catalytic activity of our compounds. As we can see from the Fig. 5 and the Table 2 the ligands substituted in para and meta position gives the best result with Cu $(CH_3COO)_2$ with a rate of 23.62 and 24.07 µmol.min^{-1}.L^{-1} successively . For the two ligands **L7** and **L8**, the shape of the curve of absorbance versus time is the same in the first 35 min, but after that, the absorbance in the presence of **L7** remains fixed during the rest of the time unlike the ligand **L8,** which decreases due to the formation of a complex in the cuvette. However, the absorbance in the presence of **L9** is weak probably due to the steric effect caused by the amino group, which prevents the (Cu^{2+}) cations from complexing better with the ligand, which is not the case in the two other ligands, where the NH$_2$ group is more far from the coordination site proposed.
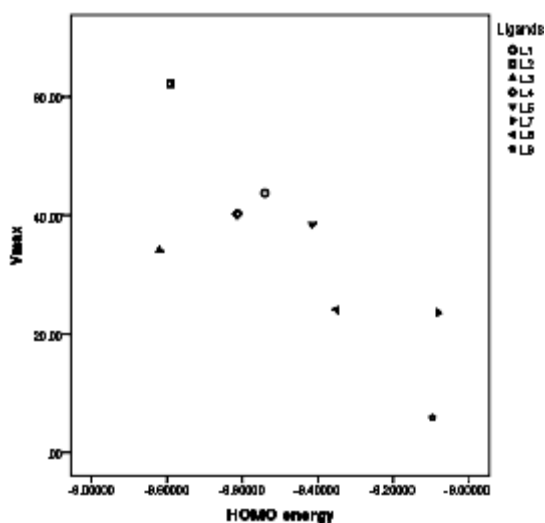

Fig. 7: Dependence of V$_{max}$ to E$_{HOMO}$

Theoretical study

In this part, we reported a theoretical study to figure out a correlation between the experimental values of Vmax (Table 3)and the calculated values of the molecular orbitals frontiers (HOMO and LUMO) obtained by the quantum mechanical calculations[14,15].

Method of calculation

Semi empirical calculations have been performed using Hyperchem program version 8.0.6 for windows[16] running on a Windows seven with a core i5 vPro. The initial geometry optimization of the studied molecules were performed with the molecular mechanics (MM+) force field[17, 18], where the lowest energy conformations are obtained. Further, Geometry optimization was done by performing the semi-empirical molecular orbital theory at the level parametric method (AM1)[19], within restricted Hartree–Fock (RHF) level[20]. The Polak–Ribier algorithm was used for the optimization[21]. The convergence is set to 0.01 kcal/mol.

Theoretical calculations

The lowest unoccupied molecular orbital (LUMO) represents the ability to accept an electron whilst the HOMO which is the highest occupied molecular orbital represents the ability to donate an electron. the gap between the HOMO and LUMO known as the energy band gap explains that eventual charge transfer interaction is taking place within the molecule. the values of the band gap (deltaE) are given in table 4. As illustrated in Fig. 6a and Fig. 6b for L1, (spatial distribution of the HOMO and LUMO of the other molecules is presented in the supplementary material (**Fig. 6c** and **Fig. 6d**)). the HOMO orbitals are localized on the benzene ring and the imine function, distribution of molecular orbitals of LUMO is localized on pyrane ring.

Correlation

A study was conducted using IBM SPSS Statistics v 21 to find a correlation between the maximal reaction rate (V_{max}) and the HOMO energy. The results were given in Table 5 and Fig. 7. Correlation quantifies the extent of the relationship two quantitative variables, here V_{max} and E_{HOMO}. When high values of V_{max} are associated with high values of E_{HOMO} a positive correlation exists. However, a negative correlation obtained, when high values of V_{max} are associated with low values of E_{HOMO}.

A strong correlation is observed between the maximal reaction rate (V_{max}) and HOMO energy, so the correlation is significant (r = -0.794; P = 0.019), here the correlation is negative.

These results show a good linear correlation between the maximal reaction rate and HOMO energy. This correlation proves the existence of a relationship between the catalytic activity, which is presented by V_{max}, and HOMO energy, so we can estimate V_{max} by calculating of the HOMO energy for this kind of ligands.

CONCLUSION

In conclusion, we have found that our compounds **L1**–**L7** present a variant capacity to catalyze the oxidation reaction of the catechol to o-quinone at ambient conditions using the oxygen of atmosphere as oxidant. The catalytic activity of these complexes formed in situ is influenced by the nature of counter anion in metallic salt, ligand and the metallic salt concentration, nature of solvent and the nature of substitutent group on the benzene ring. In addition, the Quantum mechanical and statistical calculations showed a good correlation between the maximal reaction rate and the HOMO energy which proves the existence of a strong relationship between the catalytic activity and the HOMO energy. It seems interesting to continue this work, firstly, on the synthesis of these complexes and testing their reactivity as metallo-enzymes in the field of biomimetic catalysis. Secondly, on trying to find a general Model of $Cu:O_2$ intermediates and understanding their action mechanism in the oxidation reaction of the catechol to o-quinone.

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