New Nickel Metal Complex towards Biomimetic Catecholase Enzyme Oxidation: Synthesis and Kinetics Studies

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Abstract: Pyrano[2,3-c]pyrazole ligand and their transition metal complex with nickel ($Ni^{II}MPC$) were synthesized and characterization by elemental analyses, magnetic susceptibility, FT IR, UV-Vis, and mass spectroscopy. Complex have been investigated as functional models for catechol oxidase activity by using a catechol as a model substrate. According to the kinetic measurement results, the rate of catechol oxidation follows first order kinetics. Nickel complex has been used in homogenous oxidation of catechol in presence of a green oxidant H_2O_2. kinetic parameters were obtained V_{max}= $2.698\times10^{-3}MS^{-1}$, K_m = 7.878M, k_{cat}= $4.496\times10^{-2}S^{-1}$ and k_{cat}/K_m = 0.5707 $M^{-1}S^{-1}$.

Keywords: Pyrano[2,3-c]Pyrazole, Nickel, Oxidation, Catecholase

1. Introduction

Metals have an esteemed place in medicinal chemistry. Transition metal complexes have been widely studied in the area of bio-inorganic and catalytic chemistry [1, 2] Metal complexes with active drugs as ligands is a research area of increasing interest for inorganic and medicinal chemistry and has concentrated much attention as an approach to new drug development [3, 4].

The oxidation of phenols compounds under moderate conditions is very important in the decay of aromatic compounds and in industrial catalysis for environmental ecology [5]

Latterly much works have been based on pyrazole ligands to form metal complex to mimic catecholase enzyme function [6-7] pyrazole ligand have identical structure of imidazole ring, the imidazole ring of amino acid remains is combined ligands at the energetic sites of metalloproteinase which guided to employ pyrazole to mimic the function of catecholase [8].

Pyrano[2,3-c]pyrazoles has been many applications in the field of pharmaceuticals [9] these compounds have biological activity and pharmacological [10] anticancer, [11, 12] anti-inflammatory, [13].

Catecholase widespread in plant enzyme, to save plant from harm result by insects and pathogens by catalytic oxidation of catechol to *o*-quinones in the presence of oxygen air [14, 15] catecholase activity mimicking by bio-materials structural, metal complexes considered in recent years the most important in a study of their catalytic activity in oxidation reactions [16] in spite of the powerful site of catecholase enzyme consists of a hydroxo-bridged dicopper(II) center, it is fully certified that many monometallic complexes of Mn(II) [17], Ni(II) [18], copper(II) [19] and Fe(II) [20] are show catecholase activity.

To more recognize the effect of concentration and nature of substrate the reaction follow up Michaelis-Menten [21] to study the kinetic of enzymatic reaction to limitation the kinetic parameters.

2. Experimental

2.1. Materials and Methods

Aromatic aldehydes and its substituted derivatives, were

purchased from Aldrich Chemical Co. malononitrile, 3-methyl-1-phenyl-2-   pyrazolin-5-one   and triethylbenzylammonium chloride (TEBA) were purchased from Across Organics Co. (Belgium). Nickel chloride was obtained from Merck Co., Germany.

2.2. Synthesis of (6-amino-4-(4-methoxyphenyl)-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile) (MPC)

When aromatic aldehydes (1), malononitrile (2), 3-methyl-1-phenyl-2- pyrazolin-5-one (3), and triethylamine were heated under reflux, the products (4) were obtained in good yields [22] The infrared spectrum of the (MPC) shows band at 3382 cm^{-1} with one at 3317 cm^{-1} assigned to νNH_2. The ligand also shows strong bands at 2190 cm^{-1}, 1511 cm^{-1} assigned to ν C=C, ν C≡N. the mass spectroscopy (m/z = 358.1) M. p. 173–1758C (Lit. [23] 170–1728C).

^{1}H NMR (DMSO-d6, d, ppm): 1.78 (3H, s, CH$_3$), 3.74 (^{3}H, s, CH$_3$O), 4.63 (^{1}H, s, C$_4$-H), 6.90 (^{2}H, d, J ¼ 8.4 Hz, ArH), 7.16–7.17 (^{4}H, m, NH$_2$ ArH), 7.30–7.34 (^{1}H, m, ArH), 7.47–7.51 (^{2}H, m, ArH), 7.78 (^{2}H, d, J ¼ 8.8 Hz, ArH).

Anal. Calcd. for C$_{21}$H$_{18}$N$_4$O$_2$: C 70.38, H 5.06, N 15.63; found C 70.54, H 4.89, N 15.40 [24]. ("Figure 1").
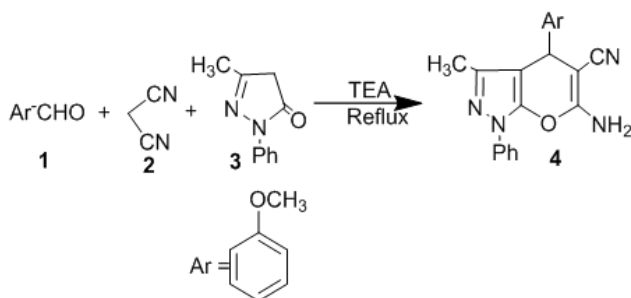

Figure 1. *Synthesis of MPC ligand.*

2.3. Synthesis of [NiMPC] Complex

Nickel (II) chloride (0.1 mole) was dissolved in ~ 40 ml absolute ethanol, then added to 0.1 mole of the prepared ligand (MPC) dissolved in ~ 40 ml absolute ethanol. The mixture was heated under reflux for two hrs. A bluish precipitate was formed that was filtered off and finally washed by hot ethanol several times.

2.4. Catecholase Activity and Kinetic Measurements of Oxidation Activities

The kinetics measurements of the reactions were studied spectroscopically the catalytic activity of the Ni^{II}MPC complex toward the homogenous oxidation of catechol in methanol solution at 25°C was determined by measuring the initial rate of catechol oxidation. The increase of the absorption at 390 nm [25] due to the oxidation product with time was obtained on a Varian Cary 3E spectrophotometer. A plot of the formation of the product with respect to time gives the initial rate. 60µM of the Ni^{II}MPC catalyst has been used in the oxidation of different concentrations of the substrate

(5–150µM) in presence of 100 µM H$_2$O$_2$ to study the effect of catechol concentration on the reaction. The rate laws were determined and rate constants obtained.

2.5. Instrumentation

Carbon, hydrogen and nitrogen contents were determined at the Micro analytical Unit, Cairo University, Egypt. IR spectra of the ligand and its solid complexes were measured in KBr on a Mattson 5000 FTIR spectrometer. The electronic spectra and kinetic measurements were performed using Varian Cary 4 Bio UV/VIS spectrophotometer. ^{1}H- NMR spectrum of the ligand was recorded on Joel-90Q Fourier Transform (200 MHz) spectrometers in [D$_6$] DMSO. The mass spectra of Thermal analysis measurements (TGA) were recorded on a Shimadzu thermo-gravimetric analyzer model TGA-50 H, using 20 mg samples. The flow rate of nitrogen gas and heating rate were 20 cm^3 min^{-1} and 10°C min^{-1} respectively. The magnetic susceptibility measurement for the nickel (II) complex was determined by the Gouy balance using Hg[Co(NCS)$_4$] as a calibrate at room temperature.

3. Results and Discussion

3.1. FT-IR

The pyrazolyl derivative was synthesized and characterized by ^{1}H NMR, IR, mas and elemental analysis [22].

By compering of FT-IR of ligand (MPC) and Ni^{II}MPC complex strong band appeared at 2195 cm^{-1} which is given to the C≡N vibrations in ligand this band was vanish in the Ni (II) complex. Shifting in νNH_2 bands to higher wavelength (3430 and 3320 cm^{-1}) and this is supported the Ni(II) bind to MPC ligand through the nitrogen atom of NH$_2$ and nitrogen atom of CN. a new band at 475 cm^{-1} due to $\nu Ni– N$ [26]. This also confirming structure by the presence of new band at 545 cm^{-1} to $\nu Ni– O$ [27].

3.2. Electronic Spectral Data

At room temperature these complexes show magnetic moment in the range 2.98 BM. These values are in tune with a high spin configuration and show the presence of an octahedral environment [28] around the Ni (II) ion in the complexes. Exhibit absorption bands in the range 260 nm and 480nm. An examination of these bands indicates that the complexes have an octahedral geometry. The ground state of Ni (II) in an octahedral coordination is $^3A_{2g}$. Thus, these bands may be assigned to the three spin allowed transitions [29]. $^3A_{2g}$ (F) → $^3T_{2g}$ (F)—(v1), $^3A_{2g}$ (F) → $^3T_{1g}$ (F)—(v2), $^3A_{2g}$ (F) → $^3T_{1g}$ (P)—(v3), Respectively these support an octahedral geometry [30].

3.3. Mass Spectrum

Nickel complex "Figure 2" shows a molecular ion peak at m/z = 537 corresponding to the nickel complex moiety

$(C_{21}H_{22}Cl_2NiN_4O_5)$. The base peak was found in the spectrum at m/z = 54 (100%) mass fragmentation pattern of metal complex shows in "Figure 3"
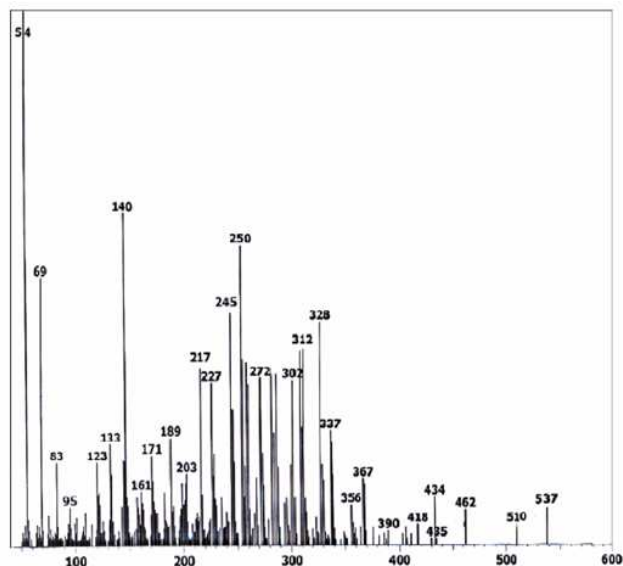

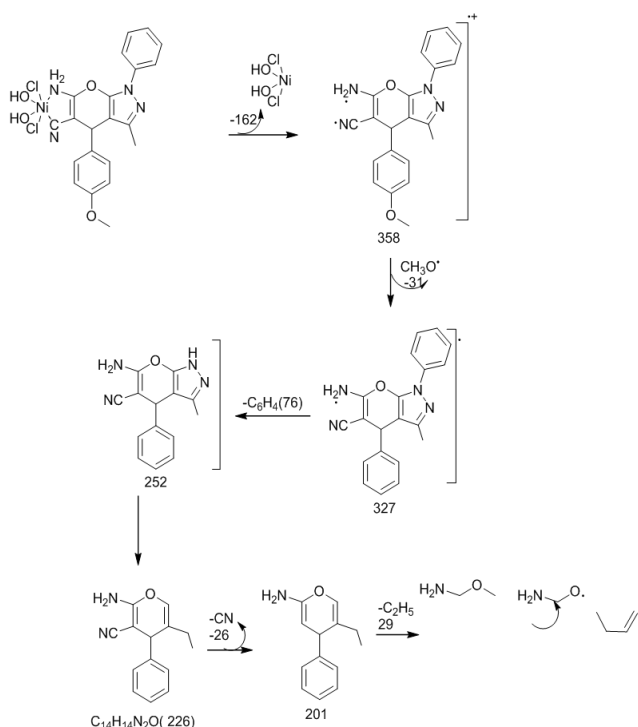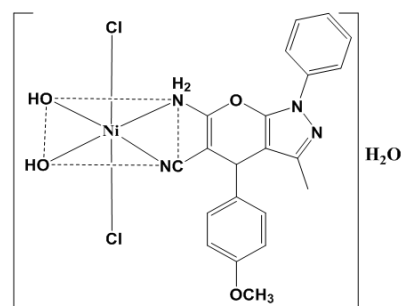**Figure 2.** *Mass spectrum of [Ni(MPC)(OH)₂Cl₂]H₂O.*

Figure 3. *Mass fragmentation pattern of metal complex.*

3.4. Elemental Analysis

Ni^{II}MPC complex shown% C (cal; 46.71, foud; 46.31)% H (cal; 4.11, Foud; 4.02)% N (cal; 10.38 foud; 10.24)% Ni (cal; 10.87 foud; 10.78).

The elemental analysis addition to IR, mass spectroscopy, magnetic moment and electronic spectral all this give the structure (1) of Ni^{II}MPC.

Structure (1) chemical structure of Ni^{II}MPC

3.5. Catalytic Activity and Kinetic Study of Catecholase

3.5.1. Effect of Concentration of Catalyst

In order to investigate the effect of the concentration of catalyst on the oxidation of catechol with different concentrations of the nickel complex Ni^{II}MPC have been used in the oxidation of catechol. The initial rate $V_i = 9 \times 10^{-4}$ was found to be linear till 60 μM and then reach a saturation stage "Figure 4"

3.5.2. Effect of Substrate

Since catechol is instantaneously oxidized by activated H_2O_2, the rate-determining step in this case is the activation of H_2O_2. Thus, catechol here serves as a "redox indicator". In order to establish the rate law for the homogeneous catechol oxidation by nickel complex, the rate is further determined at various concentrations of catechol at 25°C in ethanol solution. The rate of catechol oxidation using 60μM complex Ni^{II}MPC against different concentrations of catechol is measured and found to be hyperbolic with respect to [catechol], suggesting the possibility of a pre-equilibrium kinetics. This kinetics can be described as the binding of the substrate catechol (S) to the metal center Ni^{II}MPC to form the intermediate Pyrano[2,3-c]pyrazole nickel complex, followed by the conversion of the bound substrate into the product. The first order rate constant k_{cat} and dissociation constant K_m are found. The small value of the K_m indicates a very good binding between the nickel complex and substrate catechol.
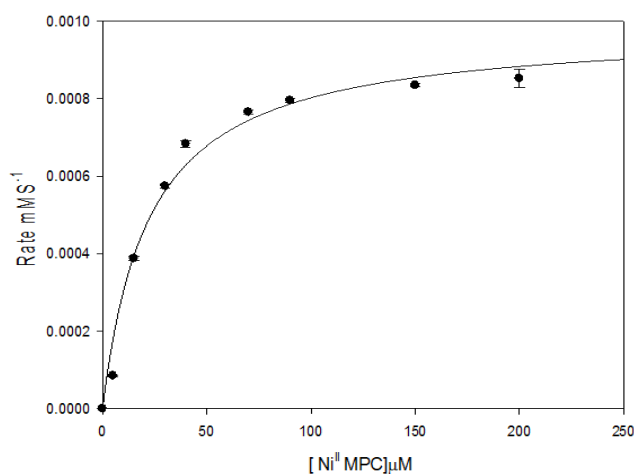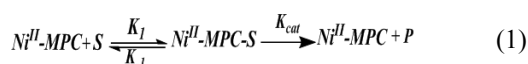

Figure 4. *Oxidation of 1mM of catechol using different concentrations of the catalyst Ni^{II}MPC at 25°C.*

The catecholase activity of the complexes was studied using catechol as substrate in ethanol at temperature 25°C is observed in catechol was determined by measuring The increase of the absorption at 390 nm with time due to the oxidation product with time was obtained "Figure 4" on a Varian Cary 3E spectrophotometer in the presence of H_2O_2 solution as an oxygen source. By applying traditional the Michaelis–Menten approach "Figure 5", values of the kinetic parameters V_{max}, K_m, k_{cat} and k_{cat}/K_m were obtained from the corresponding plot of Vi versus [S] (different concentration catechol) in "Figure 6" V_{max}= 2.698×10^{-3}, K_m = 7.878M, k_{cat}= $4.496 \times 10^{-2} S^{-1}$. The reaction in concentration of catalyst $Ni^{II}MPC$ complex 60 μM and in the presence of saturation amount of H_2O_2 (200 μM). produces important catalytic efficiency k_{cat}/K_m = 0.5707 $M^{-1}S^{-1}$ as second order rate constant.

$$Ni^{II}\text{-}MPC + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} Ni^{II}\text{-}MPC\text{-}S \overset{K_{cat}}{\longrightarrow} Ni^{II}\text{-}MPC + P \qquad (1)$$

$$rate = \frac{K_{cat}\,[Ni^{II}\text{-}MPC][S]}{Km + [S]} \qquad (2)$$

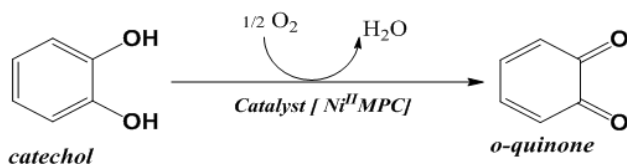$$V_{max} = K_{cat}\,[Ni^{II}MPC] \qquad (3)$$

Figure 5. Oxidation of catechol to o-quinone.

3.5.3. Effect of Concentration of Hydrogen Peroxide

The rate of oxidation of catechol at various Concentrations of hydrogen peroxide using 60 μM of the catalyst catechol complex shows saturation pattern at 200μM "Figure 7". This saturation behavior indicates that the hydrogen peroxide binds to the nickel complex or nikel complex banded to the substrate catechol.
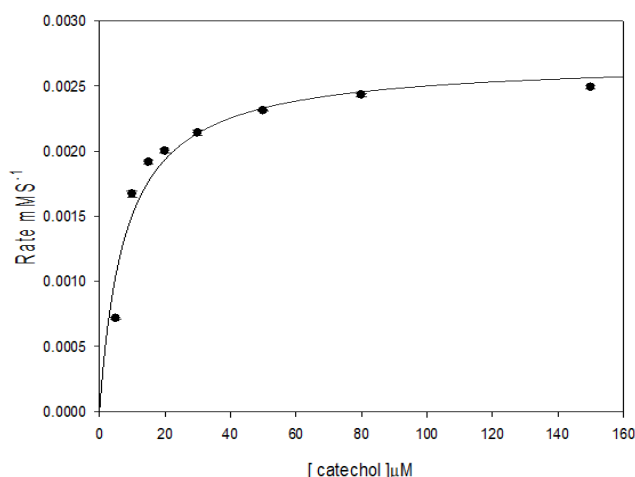

Figure 6. Plot of initial rate (mMS^{-1}) VS [Substrate] μM of catechol in presence of catalyst complex Ni^{II}MPC 60 μM at 25°C.
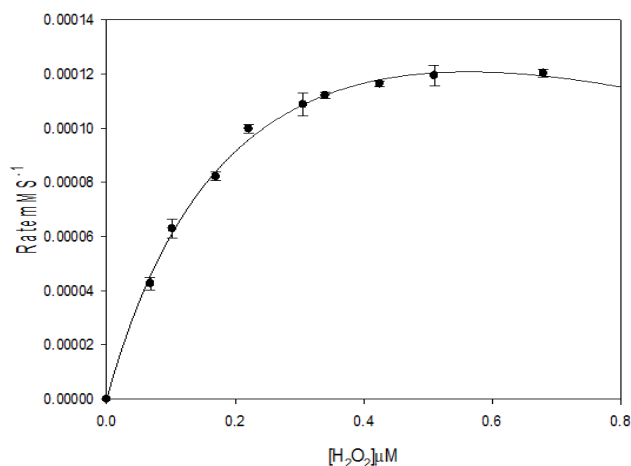

Figure 7. Oxidation of 1.0 mM catechol using 60 μM Ni^{II}MPC with different concentrations of H$_2$O$_2$ at 25°C.

4. Conclusion

In this studies a new complex of Nickel was synthesized with pyrano[2,3-c]pyrazoles This complex was fully characterized by different tools (FT-IRI, Electronic spectral data, magnetic moment, Mass spectrum, Elemental analysis) The Ni(II) bind to MPC ligand through the nitrogen atom of NH_2 and nitrogen atom of CN. The geometry around the Nickel ion is found to be octahedral geometry. In this study The [Ni^{II}MPC] complex has been used as a catalyst in the oxidation of catechol in presence of H_2O_2 as a green oxidant and this reaction biomimetic enzyme reaction and study the Effect of concentration of catalyst and Calculates The initial rate V_{i}, study the effect of substrate (catechol) and Calculates the kinetic parameters V_{max}, K_m, k_{cat} and k_{cat}/K_m. in the end study Effect of concentration of hydrogen peroxide in oxidation of catechol.

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