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# Oxidase Studies of Substituted Catechols using Copper (II) Complex of Bis (2-benzimidazolylmethyl) ether

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**Abstract:** ***The synthesis of a Cu(II) complex [Cu(DGB)$_2$(ClO$_4$)(CH$_3$COO)] of Bis (2-benzimidazolylmethyl) ether has been described. The copper (II) complex use as catalyst to carry out the oxidation of substituted Catechols (DTBC) in methanol using molecular oxygen as the oxidant.spectroscopic method has been used to determine the rate of reaction. The initial rate of formation of the product is found to be dependent on the amount of substrate employed. In the presence of dopamine and 3,4-dihydroxy benzoic acid the catalyst do not show any catalysis. It means that the presence of dopamine and 3,4-dihydroxy benzoic acid block the active sites of catalyst, which is responsible for the catalysis.***

**Keywords:** Benzimidazole, Copper Complex, Catechol Oxidation, Kinetic Study

## 1. Introduction

Interest in the use of chelating ligands incorporating benzimidazole as models for the active sites in several copper containing proteins has also grown rapidly since imidazole was identified as one of the coordinating ligands in many of the copper containing proteins [1]. Catechol oxidases are enzymes that possess a dinuclear copper center in their active site, and the copper centre is known to be coordinated by three N- atoms from three histidines [2-5]. This enzyme catalyzes the substrate specific oxidation of o-diphenols to o-diquinones in plants [6,7]. The copper centres in this enzyme are bound to imidazole nitrogen of histidine and have a nitrogen-oxygen donor environment. Currently a lot of research has been devoted in the preparation of non-toxic synthetic models of catechol oxidase [8]. It has been proposed that ligand flexibility is an essential condition for the reactivity of copper complexes and geometric factors are important in understanding the difference in the reactivity of copper (II) compounds [9]. Further it is necessary to have free coordination site on the copper (II) coordination compound for weak binding of the catechol. Therefore, ligands having small number of donor atoms are important in generating the functional mimics of catechol oxidase. The ligating system DGB provides benzimidazole nitrogen and oxygen as a donor group and generates an opportunity to synthesize Cu(II) complexes having functional similarities to the above-mentioned enzyme.

## 2. Experimental

Bis (2-benzimidazolylmethyl) ether (DGB) was prepared as described earlier [10]. Freshly distilled solvents were employed for all the synthesis. All other chemicals were of AR grade. Spectroscopic studies were carried on a Shimadzu 1601 spectrometer at the Department of Chemistry, University of Delhi, Delhi.

### 2.1 Preparation of Ligand (DGB)

Preparation of $O(CH_2C_7)_4N_2)_2$ (DGB). A 12.1-g (90-mmol) amount of diglycollic acid was combined with 19.5 g (180 mmol) of o-phenylenediamine and powdered. The mixture was heated for about 3h at a temperature of about 150 ºC on a oil bath, till all effervescence ceases. The resulting red-blue glasslike solid after cooling was powdered and was added to 250 mL of 4 M HCl in small amounts. Upon scratching of the sides of the flask, a gray-white precipitate was obtained. This was filtered out and washed by slurrying in acetone several times. The above hydrochloride was dissolved in 200 mL of distilled water, and the resulting filtrate was then neutralized with 1:1 ammonia until a white precipitate was formed. This white precipitate was collected, washed with ether, and recrystallized from acetone: yield 14 g; mp 280-295 ºC.

### 2.2 Preparation of complex containing the ligand DGB and exogenous ligand acetate.

**[(DGB)$_2$(ClO$_4$)(CH$_3$COO)Cu·2H$_2$O]** [10]

The complex was prepared as described earlier. The ligand (0.66 mmol) was suspended in acetonitrile, and copper perchlorate (0.33mmol) solution (5 mL) was added to the ligand suspension. Most of the ligand goes into solution. A 0.33mmol portion of sodium acetate, and benzimidazole was added to each of the respective solutions. The blue color of the solution changes to pinkish violet in acetate. After 20 min of stirring, a precipitate is formed. The resulting crude product was then washed with acetonitrile and recrystallized from a $CH_3CN$-DMF mixture.

### 2.3 Catechol oxidase studies

A mixture of 4.0 ml of 3,5-ditertiary-butylcatechol (25 mM) (saturated with dry nitrogen) copper (~/1 mM) in methanol (saturated with dry dioxygen) was placed in a 1 cm path length optical cell in a spectrophotometer. The formation of o-benzoquinone was followed by observing the increase of characteristic quinone absorption band in the range 390–/410 nm. Absorbance Vs wavelength plot (figure 1) shows increase in Absorbance band at 410 nm. Blank run for 3,5-ditertiary-butylcatechol, was also performed in the same

manner by adding 2.0 ml of oxygen saturated methanol to 2.0 ml of 3,5-ditertiary-butylcatechol, solution (25 mM) in nitrogen saturated methanol. Similarly blank run for complex was also performed. The conc. vs time plot by varying the conc. of substrate in different ratio are shown in figure 2.

The same experiment was repeated using dopamine and 3,4-dihydroxybenzoic acid, but no catalysis is observed in these case.

For each set of observation, a curve of concentration of o-benzoquinone formed (calculated by using corresponding ε value) versus time was plotted and initial rates were calculated by drawing a tangent to curve at t=0 and finding its slope. After this initial fast phase, the average rate of reaction was also calculated.

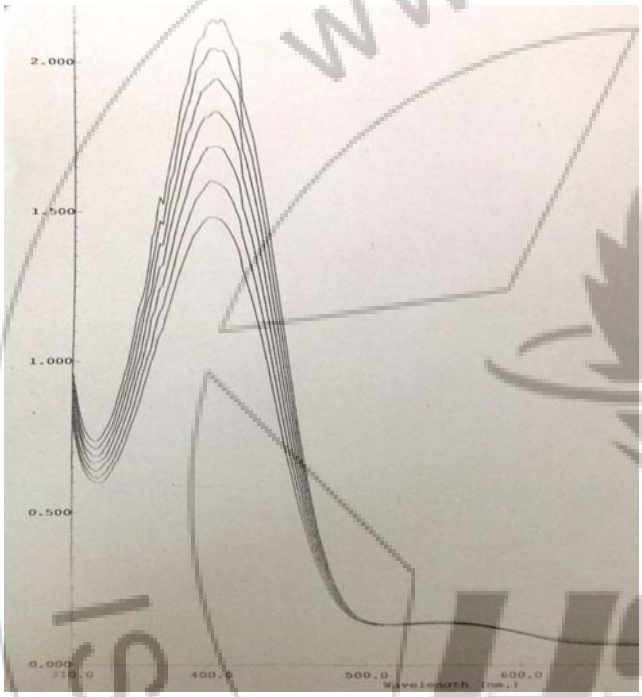


**Figure 1:** Absorbance Vs wavelength plot shows increase in Absorbance band at 410 nm.

### 2.4 Kinetic studies of the reaction of $[(DGB)_2(ClO_4)(CH_3COO)Cu]$ with 3,5-ditertiarybutyl catechol

Concentration of DTBC was varied (10, 15, 20, 25 mM) and concentration of catalyst was kept fixed at 1 mM. In the reaction mixture the concentration of catalyst $[(DGB)_2(ClO_4)(CH_3COO)Cu]$ was varied (0.5, 1.0, 1.5 mM) and concentration of DTBC was kept fixed at 25 mM. The conc. vs time plot by varying the conc. of catalyst in different ratio are shown in fig. 3

For each set of data initial rates were calculated and graphs of rate versus concentration of catalyst and rate versus concentration of catechol were plotted (figure 4)

The catalytic oxidation of various catechols and in particular 3,5-di-tert-butylcatechol (DTBC) has been widely studied as a model reaction for the catecholase activity of tyrosinase [11,12]. We have utilized the complex $[(DGB)_2(ClO_4)(CH_3COO)Cu]$ for such studies and it has been found to catalyse the oxidation of various o-dihydroxybenzenes like 3,5-di-tert-butylcatechol (DTBC), dopamine (DOP), 3,4- dihydroxybenzoic acid (3,4-DHB).

The solvent employed for all the oxidations was methanol. A 25 mM solution of catechol displays no band in the region 300-900 nm. $[(DGB)_2(ClO_4)(CH_3COO)Cu]$ however displays a charge transfer band at 378 nm. A 4.0-ml mixture of catechol (25 mM) and $[(DGB)_2(ClO_4)(CH_3COO)Cu]$ (1 mM) is prepared and the absorption spectrum was immediately scanned in the region 300-1100 nm. For DTBC, the quinone production exceeds beyond the stoichiometric amount.

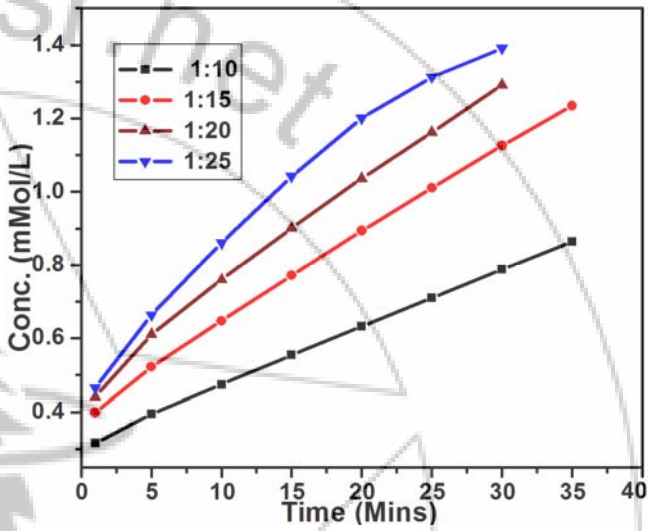


**Figure 2:** The conc. vs time plot by varying the conc. of substrate in different ratio.

With DOP and 3,4-DHB, no quinone generation could be observed. For DTBC the concentration of corresponding quinone produced was calculated [12a] using ε= 1585 $M^{-1}$ $cm^{-1}$. The reaction rates have been calculated. Initial rates have been calculated by drawing a tangent to the curve at t=0. For o-diphenol initial concentration is 25 mM and for .

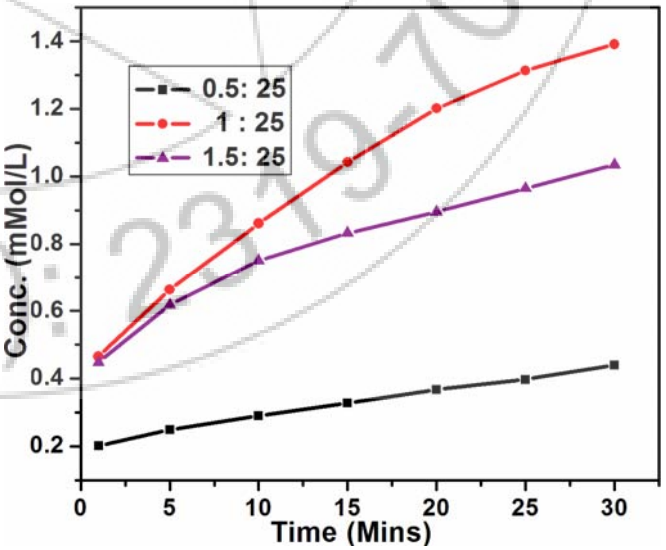


**Figure 3:** The conc. vs time plot by varying the conc. of catalyst in different ratio

$[(DGB)_2(ClO_4)(CH_3COO)Cu]$ initial concentration is 1 mM, the initial rates were found to be as shown in table 1. Table 1 gives the conversion of various catechols to quinones at 30 min. The rates though not comparable to the copper enzyme, tyrosinase, are found to be quite higher than other synthetic complexes [12c]. The activity is found to vary with the substituent on catechol as $HOOC\sim CH_2CH_2NH_2 < /CH_3 < /t\text{-}Bu$. The order of reactivity shows that the o-diphenol where the substituent is the best electron-donating group gives the highest activity. Similar trends are found by other groups [12b] also both for synthetic complexes and for the enzyme tyrosinase. These results are consistent

**Table 1:** Rate of the reaction in different ratio

| *Ratio* | *Rate of a reaction*$10^{-2}$ $mML^{-1}min^{-1}$* |
|---|---|
| 1:10 | 1.59 |
| 1:15 | 2.48 |
| 1:20 | 3.18 |
| 1:25 | 3.96 |
| 1.5:25 | 3.02 |
| 0.5:25 | 0.91 |

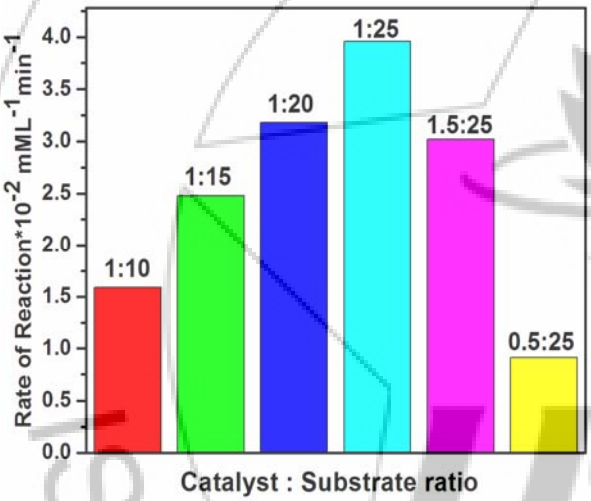


**Figure 4:** Comparsion of rate in different ratio of catalyst: Substrate

with the catecholase activity, which is dependent upon the formation of a Lewis acid-/base bond between copper (II) and o-diphenol oxygen atom. Based on this, absence of any quinone formation for 3,4-DHB could be understood in terms of a greater electron withdrawing effect of the carboxyl moiety, i.e. the COOH group in this particular catechol. It has been reported earlier that for a single mononuclear complex, the rate of DTBC oxidation depends linearly on the square of the copper (II) concentration[15] while our results indicate that it depends only on the first power of catalyst concentration. This implies that in the present series of complexes, the possibility of catechol bridging the two copper(II) centres is remote. This is quite likely due to the rigidity of bulky benzimidazole groups that may hinder the formation of such bridged species with catechol. Thus, the rate determining step involves only a single metal centre. Such an observation is not uncommon [13].

## 3. Conclusion

In the present paper the synthesis of Bis (2-benzimidazolylmethyl) ether ligand and its copper(II) complex has been demonstrated. The ligand behaves like tridentate chelating ligand with 2N and O donor sides. The catalytic properties of the copper (II) complex have been evaluated for catechol oxidation in the presence of dioxygen, under mild and environmental friendly operating conditions. Homogeneous systems were found to be effective catalyst for oxidative conversion of DTBC to DTBQ. Apart from this catalytic studies were also carried with dopamine and 3,4-dihydroxy benzoic acid. But the analysis shows that the oxidation does not take place with these substrate under similar condition. The variation in the substrate and catalyst concentrations, affects the rate of reaction upto the certain extent. They show optimum rate of reaction in the ratio of 1:25, beyond that that the rate of reaction decreases.

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