CHAPTER VIII
ANTIBACTERIAL AND CATALYTIC ACTIVITY STUDIES ON SOME COBALT(II) AND COPPER(II) COMPLEXES OF 1-NITROBENZYL-2-NITROPHENYLBENZIMIDAZOLE

Apart from our work on the synthesis and characterization of some new metal complexes, a study was also undertaken to know the catalytic activity, antimicrobial activity and thermal diffusivity of some of the complexes reported in this thesis. In this chapter, we describe the results of our studies on the antimicrobial and catalytic activity of the complexes of cobalt(II) and copper(II) derived from NBPBI.

This chapter comprises of two sections, Section A and Section B. Antibacterial activity studies of the simple complexes of cobalt(II) and copper(II) derived from 1-nitrobenzyl-2-nitrophenylbenzimidazole (NBPBI) are described in Section A of this chapter. Section B deals with the catalytic activity studies of both simple and polymer supported cobalt(II) and copper(II) complexes derived from NBPBI. The simple complexes of cobalt(II) and copper(II) have been screened for their catalytic activity in the oxidation of 3,5-ditertiarybutylcatechol (Part I), and that of the polymer supported complexes in the decomposition of hydrogen peroxide (Part II).

The thermal diffusivity of the simple and polymer supported complexes of cobalt(II) and copper(II) derived from NBPBI have been carried out in collaboration with the International School of Photonics of our University, and the results of this work have very recently been reported elsewhere\textsuperscript{1,2}. The studies show that the polymer supported complexes have higher values of thermal diffusivity than the corresponding simple complexes.
INTRODUCTION

Benzimidazole derivatives exhibit useful pharmacological activities and these increase over many folds when these compounds are complexed with metal ions. The antimicrobial activity and other medicinal applications of some transition metal complexes have been reported in the recent literature. This observation prompted us to study the antibacterial activity of the ligand, NBPBI and its cobalt(II) and copper(II) complexes towards both Gram positive and Gram negative bacteria, the results of which are presented here.

EXPERIMENTAL

Materials and methods

Details regarding the synthesis and characterization of simple complexes of cobalt(II) and copper(II) derived from NBPBI are given in Chapter III.

The antibacterial activity of the complexes of cobalt(II) and copper(II) derived from NBPBI was determined by assessing the rate of growth of selected Gram negative (G-ve) and Gram positive (G+ve) bacteria in the presence of the prepared complexes in growth medium, as described below.
**Source and maintenance of bacteria**

Microbial strains used in the present study included G-ve *Escherichia coli* (CBT GS 109), *Vibrio costicola* (ACMR 267) and *Pseudomonas flourescence* (ACMR 171), and G+ve *Bacillus polymyxa* (CBTB 25) and *Streptoverticillium sp.* (CBT SA 133). They were obtained from the culture collection of the Department of Biotechnology, Cochin University of Science and Technology, Kochi, India. *E. coli*, *V. costicola* and *B. polymyxa* were maintained on nutrient agar (HiMedia, India) while *Streptoverticillium sp.* was maintained on Bennet's agar (HiMedia, India) slants.

**Inoculum preparation**

*B. polymyxa*, *V. costicola* and *P. flourescence* were grown in nutrient broth (HiMedia, India). *E. coli* was cultured in Luria broth (HiMedia, India) and *Streptoverticillium sp.* was grown in glucose-peptone-yeast extract-beef extract broth. The following procedure was adopted for the preparation of inoculum.

(i) A loopful of 18-24 h old culture was transferred aseptically to 10 mL of the respective sterile media taken in test tubes, and incubated at room temperature (28 ± 2°C) for 24 h on a rotary shaker at 150 rpm. In the case of *Streptoverticillium sp.* 4 day dd slant culture was used as inoculum and the inoculated medium was incubated for 48 h.
(ii) After incubation for the said period, the culture grown in the test tube was added to 100 mL of sterile medium respective of each test strain, and incubated further for 24 h (48 h for *Streptoverticillium sp.*) at room temperature (28 ± 2 °C) on a rotary shaker (150 rpm).

(iii) After the growth, the cells were harvested by centrifugation at 10,000 rpm for 15 minutes in a refrigerated high speed centrifuge (Kuboto, Japan), washed twice with sterile physiological saline (NaCl, 0.85 %) and resuspended in the same. This prepared cell suspension was adjusted to a cell concentration of 10^8 cells per mL.

*Effect of the complexes of cobalt(II) and copper(II) derived from 1-nitrobenzyl-2-nitrophenylbenzimidazole on the bacterial growth.*

Different dilutions of the complexes ([CoL_2Cl_2], [CoL_2Br_2], [CoL_2I_2], [CuL_2Cl_2] and [CuL_2Br_2], where L = NBPBI) in the range 100 µg/mL to 500 µg/mL were prepared from a stock solution containing 1 mg/mL in DMF, added to 5 mL of the respective media taken in test tubes, sterilized by autoclaving at 121 °C for 15 minutes, cooled to room temperature and uniformly inoculated with the prepared inoculum at 1.5 % for *E. coli, B. polymyxa, V. costicola* and *P. flourescense*, and at 4 % for *Streptoverticillium sp.* The inoculated tubes were incubated on a rotary shaker at 150 rpm for 24 h / 48 h at room temperature. Growth was determined by measuring the turbidity at A_600
using a Shimadzu 160A UV-Visible spectrophotometer. Appropriate control tubes were also maintained for all the complexes at all the dilutions tested.

RESULTS AND DISCUSSION

The simple complexes of cobalt(II) and copper(II) derived from 1-nitrobenzyl-2-nitrophenylbenzimidazole (NBPBI) were tested for their antibacterial activity against both G+ve and G-ve bacteria. All complexes showed inhibition of growth of all the strains in a linear fashion, along with an increase in concentration from 100 μg/mL to 500 μg/mL (Table I). Maximal inhibition of growth was observed at 500 μg/mL concentration. The percentage of growth inhibition varied between 88-91 % for E. coli, 89-93 % for B. polymyxa, 93-96 % for V. costicola, 85-89 % for P. flourescence and 55-71 % for Streptoverticillium sp. for all complexes, [CoL₂Cl₂], [CoL₂Br₂], [CoL₂I₂], [CuL₂Cl₂] and [CuL₂Br₂]. Interestingly, [CoL₂Cl₂] and [CoL₂I₂], were observed to effect inhibition of growth around 50 % of P. flourescence. Among the strains, V. costicola was more sensitive to the complexes tested. Unlike other strains, B. polymyxa was sensitive to the complexes tested only when the concentration was 500 μg/mL. The 19-25 % inhibition observed with this strain at 300 μg/mL was very low compared to other strains, which showed inhibition around 50 %. An increase in the concentration from 300 μg/mL to 500 μg/mL did not show any significant effect on Streptoverticillium sp., unlike in others.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Conc. µg/mL</th>
<th>E. coli</th>
<th>B. polymyxa</th>
<th>V. costicola</th>
<th>P. fluorescence</th>
<th>Streptoverticillium sp.</th>
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<td></td>
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<td>68.00</td>
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<td>93.00</td>
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<td>96.00</td>
<td>48.00</td>
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<td>42.00</td>
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<tr>
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<td>+7.00</td>
<td>+6.00</td>
<td>22.00</td>
<td>43.00</td>
</tr>
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</table>

DMF was used as a control and showed nil activity against the above microorganisms.
The antibacterial activity of the ligand, NBPBI was also studied. The ligand did not show any significant effect on *E. coli*, *V. costicola* and *B. polymyxa*. However, *P. flourescence* and *Streptoverticillum sp.* had inhibited growth at 42-43 % and 19-22 %, respectively, at 500 µg/mL of the ligand.
INTRODUCTION

Transition metal complexes have wide applications in the field of catalysis which mainly include the conversion of organic molecules to products of pharmacological interest, synthetic chemicals, and so on. Liquid phase oxidation of catechol and its derivatives to the corresponding benzoquinone using metal complex catalysts have been reported by several workers. Further, a few Schiff base complexes of cobalt(II) have been used for the oxidation of DTBC.

Catalytic activity of the complex, bis(acetate)-bis(imidazole)copper(II) and bis(valproato)bis(imidazole)copper(II) toward the aerial oxidation of DTBC to 3,5-ditertiary-butyl-α-benzoquinone have recently been reported, and a dinuclear copper(II) complex was also reported to exhibit catalytic activity in this reaction.
Studies on the oxidation of DTBC by FeCl₃ in THF-pyridine solvent has recently been published, and the formation of the intermediate iron(II)-3,5-ditertiary-α-semiquinone (DTBSQ) species, which is responsible for the reaction with oxygen, has been detected by EPR and electronic spectroscopy. Furthermore, some iron(III) complexes derived from benzimidazole ligands have been reported to catalyse this oxidation reaction. This kind of studies would provide new information on the activation of oxygen by metal ions, a subject of interest to synthetic organic chemists. In view of these, we have made an attempt to study the catalytic activity of the simple NBPBI complexes of cobalt(II) and copper(II). Oxidation of DTBC (Figure 1) was chosen for this purpose, as the formation of the oxidation product, ditertiarybutylquinone, can be easily followed by noting the absorbance at 400 nm (Quinone exhibits a characteristic absorption peak at this wavelength). The results of our studies on the catalytic

![Chemical structure](image)

Figure 1. Oxidation of DTBC in the presence of catalyst
activity of simple complexes of cobalt(II) and copper(II) in the oxidation of DTBC are presented in this part.

EXPERIMENTAL

Materials

Details regarding the syntheses of the complexes are described in Chapter III. The stock solutions of the complexes ($10^{-3}$ M) and that of DTBC ($10^{-2}$ M) were prepared afresh before each experiment by dissolving solid samples in triethylamine-DMF mixture (1.5 %). The concentration of DTBC in the reaction mixtures was maintained at $1.0 \times 10^{-3}$ M and that of the catalyst at $1.0 \times 10^{-4}$ M. The weights of the catalysts per 100 mL of the reaction mixture are given in Table II. The presence of triethylamine in DMF was required to render the latter alkaline.

Catalytic experiments

All kinetic runs were carried out at a constant temperature of $30 \pm 0.1$ °C in 1.5 % triethylamine-DMF mixture. The reaction was initiated by transferring calculated amount of the complex solution into the reaction flask. The reaction is monitored by following the absorbance of the product, ditertiarybutylquinone, at 400 nm. A solution of the same amount of the catalyst in the same solvent was used as blank. The absorbance measurements were made by extracting
about 3 mL of the reaction solutions at regular time intervals of 1 minute. A Shimadzu double beam spectrophotometer, model UV 160A with 1 cm quartz cells is used for absorption measurements. The concentration of the product formed was obtained from the absorbance data using a molar absorption coefficient of 2818 at 400 nm. The initial rates of the reaction were obtained by fitting the concentration versus time data into a polynomial of the form,

\[ c = a_1 + a_2 t + a_3 t^2 + \ldots \]

and obtaining the slope of the curve at \( t = 0 \). A software called "Axum" (Trimetrix, 1989) was used for this purpose. All the kinetic results were found to be reproducible within an error of ± 5 %.

RESULTS AND DISCUSSION

The rate of oxidation of DTBC in the presence of the complexes are given in Table II. These catalytic experiments suggest that all these complexes can act as catalysts in the above reaction.

On the basis of the results obtained, it may be inferred that the chloro complex of cobalt(II) has greater activity than the corresponding bromo and iodo complexes. Thus, the rate of the reaction varies in the following order:

\[ [\text{CoL}_2\text{Cl}_2] \succ [\text{CoL}_2\text{Br}_2] \succ [\text{CoL}_2\text{I}_2] \]
On the contrary, in the case of the copper(II) complexes, the bromo complex has better activity than the corresponding chloro complex. A comparison of the activities of the cobalt(II) and copper(II) complexes was also made and the studies indicate that the cobalt(II) complexes are better catalysts than their copper(II) analogues in the oxidation of DTBC.

Table II

The rate of oxidation of 3,5-diteriarybutylcatechol in the presence of the simple complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Weight of the complex taken (mg/100 mL)</th>
<th>Initial rate x 10^7 (M s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CoL_2Cl_2]</td>
<td>89</td>
<td>8.94</td>
</tr>
<tr>
<td>[CoL_2Br_2]</td>
<td>97</td>
<td>5.27</td>
</tr>
<tr>
<td>[CoL_2I_2]</td>
<td>106</td>
<td>4.57</td>
</tr>
<tr>
<td>[CuL_2Cl_2]</td>
<td>89</td>
<td>0.75</td>
</tr>
<tr>
<td>[CuL_2Br_2]</td>
<td>98</td>
<td>1.67</td>
</tr>
</tbody>
</table>

L = NBPBI
INTRODUCTION

The decomposition of hydrogen peroxide is often employed as a standard reaction to determine the catalytic activity of a polymer metal complex. Metals having stable oxidation states that differ by one (manganese(II), iron(III) and copper(II)) are very effective catalysts for the decomposition of hydrogen peroxide. Such metal ions function both as an oxidising and as reducing agent in the catalysed decomposition mechanism. Furthermore, some Schiff base complexes of metal ions like manganese(III) and iron(III) have been reported to exhibit catalytic activity in the decomposition of hydrogen peroxide. However, reports on the use of polymer supported metal complexes as catalysts towards decomposition of hydrogen peroxide are rather scanty. We have studied the catalytic activity of the polymer supported complexes of cobalt(II) and copper(II) derived from NBPBI in the decomposition of hydrogen peroxide, and the results of our studies are presented in this part.

EXPERIMENTAL

Materials

The syntheses of the polymer supported complexes of cobalt(II) and copper(II) are already described in Chapter IV. Hydrogen peroxide (Merck, 30 %) was made 10 % with ion free water.
Catalytic experiments

The experimental set-up is as shown in Figure 2. The following procedure was adopted to study the decomposition of hydrogen peroxide.

The reaction vessel containing H₂O₂ solution (10%, 20 mL) and magnetic paddle was kept at room temperature (28 ± 2 °C). The polymer supported complex (2-2.5 mg) was taken in a plastic float and was placed over the solution. The gas burette was filled with potassium permanganate solution for easy detection of the levels, and was

Figure 2. Experimental set-up for H₂O₂ decomposition study
attached to the reaction vessel. Then the vessel was tightly closed and the levels in the two arms of the gas burette were made equal, and the reading was noticed. A stop watch was started at the moment when the polymer supported complex was introduced into the $\text{H}_2\text{O}_2$ solution being stirred by a magnetic stirrer. As the reaction proceeds, oxygen gas is produced, and the level in the right arm is lowered. This was balanced by running away the solution through the tap. Readings were recorded at an interval of 2 minutes after making the levels in the two arms equal. Computations were done to find the initial rate of the reaction$^{47}$, as has been described in Section B.

RESULTS AND DISCUSSION

The rate of decomposition of hydrogen peroxide in the presence of the polymer supported complexes are as shown in Table III. The catalytic experiments suggest that all these complexes act as catalysts towards this decomposition reaction.

In the case of the polymer supported cobalt(II) complexes, the bromo complex has better activity than the corresponding iodo and chloro complexes. The rate of the reaction was found to vary in the following order:

$$\text{P-CoL}_2\text{Br}_2 > \text{P-CoL}_2\text{I}_2 > \text{P-CoL}_2\text{Cl}_2$$

But, in the case of the polymer supported copper(II) complexes, the chloro complex exhibits greater activity than the corresponding bromo complex.
Table III

Rate of decomposition of hydrogen peroxide in presence of polymer supported complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Weight of the complex taken (mg)</th>
<th>Initial rate x 10 (mL s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-CoL₂Cl₂</td>
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<td>1.16</td>
</tr>
<tr>
<td>P-CoL₂Br₂</td>
<td>2.3</td>
<td>1.85</td>
</tr>
<tr>
<td>P-CoL₂I₂</td>
<td>2.3</td>
<td>1.53</td>
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<tr>
<td>P-CuL₂Cl₂</td>
<td>2.2</td>
<td>1.79</td>
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<tr>
<td>P-CuL₂Br₂</td>
<td>2.5</td>
<td>1.39</td>
</tr>
</tbody>
</table>

P = Polymer supported Schiff base and L = NBPBI
REFERENCES


13. E. W. Ainscough, A. M. Brodie, J. D. Rand ford and J. M. Waters, 


