CHAPTER -IV
A simple and green analytical method for the determination of copper oxychloride in micellar media

SUMMARY

A simple, inexpensive and ecofriendly specrophotometric method for the determination of copper oxychloride has been described. Method is based on its catalytic effect on the redox reaction between methylene blue and sodium borohydride in anionic micellar media. The reaction is monitored photometrically by measuring the decrease in absorbance of methylene blue at 664 nm. The reagents and manifold variables influences on the sensitivity were investigated and the optimum conditions were established. Under optimum conditions, the proposed method allows the determination of copper oxychloride in the concentration range of 0.008-0.08 µg mL$^{-1}$ with detection limit of 0.002 µg mL$^{-1}$. This method is free from most of the interferences. The proposed method has been successfully applied for the determination of copper oxychloride in fruits and soil samples with satisfactory results.
INTRODUCTION

Copper is a widely micronutrient element in biological systems which is a pollutant when present in excess. It is required for the activation of dioxygen, which is essential for the survival of all living organisms. Copper has multiple functions, for iron absorption, haemopoiesis, in various enzyme activities and in the oxidation–reduction process [1]. It is one of the important and essential nutrients for human health as well as the growth of animals and plants and is widely distributed and performs diverse functions in plants and animals. It is an essential trace metal for animals and man because it is required for the formation of erythrocytes and hemoglobin as well as oxidative enzymes [2]. It combines with certain proteins to produce enzymes that act as catalysts to help a number of body functions. It also helps to provide energy required for biochemical reactions.

Copper is widely present in nature in the elemental state, in sulfides, arsenites, chlorides, and carbonates. It is introduced to the environment from natural sources and as a result of human activity. However, it is still a potentially toxic metal that can exert an inhibitory effect biological system at higher concentrations, which is the basis for formulation of copper-containing fungicides [3]. The most common compounds used as protective fungicides are copper oxychloride, copper oxide and hydroxides. The use of these compounds as fungicides has been restricted to its protective effects. Copper oxychloride is probably the most widely employed copper fungicide for a wide range of plant pathogenic fungi. It is a bluish-green insoluble powder consisting of basic copper chloride \[3\text{Cu(OH)}_2\cdot\text{CuCl}_2\], and its fungi toxicity depends on solubilization and release of ionic copper [4]. Despite the production of a wide variety of synthetic organic fungicides, copper oxychloride still predominate the field of fungicidal plants disease control and its wide use is also due to its general low toxicity to humans [5].

Copper oxychloride is widely used as foliar sprays against fungal diseases in a number of crops. It is applied annually to vineyards as a fungicide to control a significant number of plant diseases [6-7]. It is used to control leaf curl, freckle, brown rot, black spot and other fungal diseases such as control downy mildew in lettuce cucumber and grapes, early blight in tomatoes and potatoes, purple blotch in onion, leaf spot in strawberry, peanut, sunflower and beans. It is also used in alleviating copper deficiency of wheat grains [8-11]. They can be applied alone or in combination with other fungicides for seed treatment, foliar application or soil drenching [12].
However, its repeated spray applications can result in accumulation of copper in soil at a low pH which may cause toxicity [13].

The toxicity of copper fungicides is due to copper’s ability to precipitate proteins and cause coagulation of the cytoplasm. Its excess may lead to changes in the liver and damage kidneys, brain tissue, coronary vessels and myocardium [14]. Copper oxychloride is harmful if swallowed, can result in nausea, vomiting, diarrhoea and abdominal pain with inflammation of gastrointestinal tract. Inhalation of dust may result in respiratory irritation. Contact with eyes and skin may result in irritation, itching, redness and blisters. Oral LD₅₀ for rats is reported to be 1440 mg/kg: exposure limit as per OSHA is 1 mg/m³ [15-16]. Another side effect of copper fungicide is stippling of fruit tissue due to direct copper injury. This results in the formation of black homogenous mass of cells that separates the stipple from the healthy tissue by definite phellogen layer, whereas darkening of blemishes due to copper appears as darkly stained flavedo tissue without the formation of phellogen layer. Excess of copper has inhibitory effect on the growth of fungal strains, which can lead to reduced auxin, gibberelin and cytokinin levels [17]. Taking into account of these important facts, development of accurate and rapid detection methods for monitoring the level of copper in environmental is necessary and indispensable.

Many analytical methods, such as inductively coupled plasma atomic emission spectrometry [18], adsorptive cathodic stripping voltametry [19], flow injection catalytic photometric method [20-21], extractive atomic absorption spectrometry [22], flame emission spectrometry [23], flame atomic absorption spectrometry [24-25], electrochemical methods [26], ion chromatography [27], anodic stripping analysis [28-29], AAS [30-31], fluorescence determination [32], electrochemical sensor [33], optical fiber reflectance sensor [34], ICP-MS [35], ICP-AES [36-37], spectrophotometric method [38-44] and kinetic spectrophotometric method [45-49] have been reported for the determination of copper.

However, a very few methods are currently available for determination of copper in copper based fungicides. In one such method, copper is determined by flame atomic absorption spectrometry using digestion procedure with sulfuric and nitric acid [50]. In another approach atomic absorption spectrophotometric method was used to determine water-soluble copper in water-insoluble copper fungicides [51]. Copper is determined spectrophotometrically in fertilizer by complex formation with neocuproine [3]. It is also determined by reaction with
diethyldithiocarbamate in micellar medium [52]. Copper oxychloride is also determined by reaction with potassium iodide in acidic medium and the liberated iodine is determined using leucocrystal violet [4].

In the present work, copper oxychloride (as copper) is determined by its catalytic action on colour bleaching of methylene blue in micellar media by sodium borohydride. It is simple, cost effective and environmental friendly. It offers advantages like rapidity, reproducibility, good selectivity and high sensitivity, which have made this method an attractive analytical tool for the determination of copper oxychloride.

**EXPERIMENTAL**

**Apparatus**

A Systronics Visiscan 167 Spectrophotometer was used for spectral measurements.

**Reagents**

All the reagents were of analytical reagent grade. Distilled water was used throughout the experiments for preparation and dilution of reagents as well as samples.

**Copper oxychloride (50% Chevur Chemicals Ltd.)**

Stock solution of 1 mg mL$^{-1}$ was prepared by dissolving 0.2 g of copper oxychloride in acetic acid. Working standard solutions were prepared by appropriate dilution of the stock.

**NaBH$_4$ (Sigma Aldrich)**

Solution of 7.0×10$^{-2}$ mol L$^{-1}$ was prepared fresh daily in ice-cold water.

**Methylene blue (Merck, India)**

An aqueous solution of 6.2×10$^{-4}$ mol L$^{-1}$ was prepared.

**Sodium dodecyl sulfate (SDS) (Hi Media Laboratories Pvt. Ltd, Mumbai)**

An aqueous solution of 3.1×10$^{-2}$mol L$^{-1}$ was prepared in distilled water.
PROCEDURE

In a 10 mL volumetric flask, 1.0 mL of 3.1×10⁻² mol L⁻¹ of SDS, 0.5 mL of 6.2×10⁻⁴ mol L⁻¹ methylene blue solution, followed by addition of a known volume of copper oxychloride solution. The solution was diluted to 8 mL with water. Then 1.5 mL of 7.0×10⁻² mol L⁻¹ sodium borohydride solution was added to the reaction mixture, followed by dilution to 10 mL with water. Time was measured just after the addition of the sodium borohydride solution. After thorough mixing a portion of this solution was transferred to a cuvette. The decrease in absorbance of methylene blue by sodium borohydride in anionic micellar medium was monitored using the “fixed time procedure” by measuring the change in absorbance at 664 nm for the first 30-180 s from the start of the reaction.

RESULTS AND DISCUSSION

Analytical characteristics

The absorption spectrum of the dye was measured spectrophotometrically at 664 nm (Fig. 1). At the optimum experimental conditions mentioned above, the calibration graph was obtained by plotting (ΔAₛ-ΔAₜ) versus copper oxychloride concentration. From the results of experiments, fixed time of 180 s was chosen as the optimal time, as it provided the best correlation coefficient and sensitivity. The calibration graph was linear in the range of 0.008-0.08 µg mL⁻¹ of copper oxychloride (Fig. 2). Molar absorptivities and Sandell’s sensitivity of copper oxychloride were found to be 3.67×10⁵ L mol⁻¹ cm⁻¹ and 0.0012 µg cm⁻² respectively.

Effect of reagent concentration

The reaction variables were optimized to maximize the sensitivity and precision of the proposed method. In the proposed reaction 0.5 mL of 6.2×10⁻⁴ mol L⁻¹ methylene blue solution and 1.5 mL of 7.0×10⁻² mol L⁻¹ sodium borohydride were required for completion of the experimental process (Fig 3-4).

Effects of surfactant on reaction rate

The effect of various surfactants like TX-100, Tween-20, SDS and CPC were tested. Best results were observed when SDS was used. This is due to the fact that methylene blue and SDS are
oppositely charged species. The micelles can accelerate the rate of reaction by increasing the effective collisions which arise from electrostatic and hydrophobic interactions between the reactant and micellar surface. Methylene blue being a cationic dye is bound to SDS by both electrostatic and hydrophobic interactions [53]while with TX-100, Tween 20 and CPC, it is bound only by hydrophobic forces. Thus in SDS micellar media there is increase in collision probability between methylene blue and NaBH₄, which results in faster reduction process.

The effect of concentration of SDS on the system was studied at different concentrations, ranging from 1.0×10⁻² - 3.8×10⁻² mol L⁻¹ of SDS. According to the results, 3.1×10⁻² mol L⁻¹ SDS was selected as the optimum working concentration (Fig. 5). Reactions which have charged species, micelles can affect the rate of reaction by increasing the effective collisions.

Effects of temperature and time

The temperature of the solution mixture was varied over the range 10–40 °C. It was found that with increase in temperature up to 25 °C sensitivity increased. Further increase in temperature caused decrease in sensitivity. Thus 25 °C was selected as optimum temperature (Fig. 6). The optimum time was found by measuring the absorbance and changing it from 30 - 300 s after the initiation of reaction. The reaction rate increase up to 180 s. At still higher time, decrease in sensitivity is observed, thus 180 s was selected.

Effect of foreign species

To study the selectivity of the proposed method, the effect of foreign ions and pesticides which are likely to interfere in the determination of copper oxychloride was studied. The tolerance limit is defined as the concentration of the added ion causing not more than ± 3% relative error in the determination of 0.04 μg mL⁻¹ copper oxychloride. The results are summarized in Table 2.

Reproducibility

Reproducibility of the method was checked by replicate determination of 0.03 μg mL⁻¹ of copper oxychloride solution. The standard deviation and relative standard deviation of absorbance values were found to be ±0.005 and 1.41% respectively. The spectral and statistical parameters are given in Table 1.
**Reaction mechanism**

Methylene blue is a cationic dye, blue in colour. When reduced it becomes colourless. In the present method, copper oxychloride acts as a catalyst [54] in reduction of methylene blue by NaBH$_4$ in anionic micellar media (Scheme A).

Metals can serve as efficient catalyst [55-57] in many redox reactions. The reduction of methylene blue by sodium borohydride in micellar medium was accelerated when copper ion is present in the system. The reason for such rate enhancement is due to the fact that it acts as substrates for the electron transfer reaction for both the reactants. NaBH$_4$ releases an electron to the metal from where methylene blue gains an electron and is reduced. Thus copper ion in copper oxychloride acts as an efficient catalyst by getting involved in the electron transfer process.

**APPLICATION**

The proposed method was used for the determination of copper oxychloride in various samples.

**Determination of copper oxychloride in fruits samples**

Different samples of fruits free from copper oxychloride were taken. The samples were thoroughly washed prior to analysis and known amount of copper oxychloride solution was added to the 10 g of mashed samples and dried in an oven for 3 h at 500 °C. It was dissolved in 5 mL of aqua regia and heated on a sand bath to dryness [58]. The residue was dissolved in dilute acetic acid and was analyzed by the proposed and reported method [52] (Table 3).

**Determination of copper oxychloride in soil samples**

Known amount of copper oxychloride was sprayed on various soil samples. The samples were weighed and washed with 5 mL concentrated nitric acid and filtered, and the filtrate was heated on a sand bath to dryness. The residue was dissolved in dilute acetic acid and analyzed by the proposed and reported method [52] (Table 3).

**Determination of copper oxychloride residue on plant surface**

Leaf samples were taken and sprayed with known amount of copper oxychloride solution. These plant materials were placed in a funnel and washed with dilute acetic acid. Washings were
collected in boiling test tube and were analyzed by the proposed and reported method [52] (Table 3).

**CONCLUSION**

The proposed method was applied to determine copper oxychloride based on its catalytic effect on the reduction of methylene blue by sodium borohydrde in micellar media, without the need for extraction and preseparation. It offers advantages like reliability, reproducibility, in addition to its simplicity and is cost effective, environmental friendly and may be developed as a sensor with some modification. The method is free from common interference and could be recommended for routine analysis. It has been successfully applied for the determination of copper oxychloride in fruits and soil samples with satisfactory results.
Scheme A: Reduction of methylene blue by NaBH₄
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Copper oxychloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>664</td>
</tr>
<tr>
<td>Limit of Beer’s Law ($\mu$g mL$^{-1}$)</td>
<td>0.008-0.08</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)</td>
<td>$3.67 \times 10^5$</td>
</tr>
<tr>
<td>Sandell’s sensitivity ($\mu$g cm$^{-2}$)</td>
<td>0.0012</td>
</tr>
<tr>
<td>Limit of detection ($\mu$g mL$^{-1}$)</td>
<td>0.002</td>
</tr>
<tr>
<td>Limit of quantification ($\mu$g mL$^{-1}$)</td>
<td>0.005</td>
</tr>
<tr>
<td>Regression equation ($y = bx + a$)*</td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>10.67</td>
</tr>
<tr>
<td>SD of slope</td>
<td>0.054</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.007</td>
</tr>
<tr>
<td>SD of intercept</td>
<td>0.005</td>
</tr>
<tr>
<td>Correlation coefficient(r)</td>
<td>0.999</td>
</tr>
</tbody>
</table>

*Concentration in $\mu$g mL$^{-1}$
Table 2

Effect of foreign ions and pesticides on reaction

<table>
<thead>
<tr>
<th>Interfering ions and pesticides</th>
<th>Tolerance limit (µg mL⁻¹)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO₄²⁻, CH₃COO⁻, Al³⁺, Mg²⁺, Na⁺, K⁺, Ca²⁺, NH₄⁺, CO₃²⁻, NO₃⁻, Cd²⁺</td>
<td>1000</td>
</tr>
<tr>
<td>chlorpyrifos, monocrotophos, parathion, dichlorvos, dinocap, cypermethrin, malathion, formaldehyde</td>
<td>1000</td>
</tr>
<tr>
<td>Zn²⁺, Hg²⁺, Sn²⁺</td>
<td>800</td>
</tr>
<tr>
<td>Fe²⁺, Mn²⁺, Cr³⁺</td>
<td>300</td>
</tr>
</tbody>
</table>

ᵃCausing(±) 3% variation in absorbance value

Table 3

Determination of copper oxychloride in various samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Copper oxychloride added (µg)</th>
<th>Proposed method</th>
<th>Report method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total copper oxychloride recovery found (µg)</td>
<td>%</td>
<td>total copper oxychloride recovery found (µg)</td>
</tr>
<tr>
<td>Grapes</td>
<td>10</td>
<td>9.68</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.46</td>
<td>97.3</td>
</tr>
<tr>
<td>Orange</td>
<td>10</td>
<td>9.58</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.28</td>
<td>96.4</td>
</tr>
<tr>
<td>Leaves</td>
<td>10</td>
<td>9.71</td>
<td>97.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.32</td>
<td>96.6</td>
</tr>
<tr>
<td>Soil</td>
<td>10</td>
<td>9.53</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.18</td>
<td>95.9</td>
</tr>
</tbody>
</table>

ᵃMean of three observations
Fig 1: Absorption spectra of the dye

Fig 2: Calibration graph for determination of copper oxychloride
Concentration of copper oxychloride: 0.04 µg mL\(^{-1}\)

**Fig 3: Effect of methylene blue concentration on sensitivity**

Concentration of copper oxychloride: 0.04 µg mL\(^{-1}\)

**Fig 4: Effect of NaBH\(_4\) concentration on sensitivity**
Concentration of copper oxychloride: 0.04 µg mL$^{-1}$

**Fig 5: Effect of SDS concentration on sensitivity**

Concentration of copper oxychloride: 0.04 µg mL$^{-1}$

**Fig 6: Effect of temperature on reaction**
REFERENCES


57. Li, H.; Zhai, Y.; *Rare Met.* **2008**, 27, 560.