$\mathcal{R}$esearch Article

# Detection of copper ions in arid and semi-arid lands using UV-visible and fluorescence spectrophotometry

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## ABSTRACT

Generally arid soils are calcareous and prone to contamination of copper due to the usage of high-intensity agriculture and copper-based pesticides which leads to toxicity of organisms present in the soil resulting in fertility loss and crop diseases. We synthesised a sensor R, having a rhodanine scaffold for sensitive and selective detection of $Cu^{2+}$ ions available in the soil. The sensor exhibited characteristic UV-Visible spectral properties and fluorometric "turn-off" emission properties towards $Cu^{2+}$ ions. As a practical application detection of $Cu^{2+}$ ions by the Sensor R adsorbed on a test filter paper system is also reported. The UV–Vis spectrum of sensor R, exhibited an absorption band at 308 nm due to the presence of a thioamide group in the rhodanine moiety and a quantitative intensity enhancement is observed only in presence of $Cu^{2+}$ ions. In the fluorescence spectra an emission band around 380 nm was observed upon the addition of copper ions and the fluorescence intensity gradually decreased with the increasing $Cu^{2+}$ concentration indicating the formation of a complex. Nuclear Magnetic Resonance ($^1$H-NMR) analysis indicated that methylene protons signal at $\delta$ 4.79 disappeared and shifted up field to appear as a broad peak at $\delta$ 3.62. Filter paper strips were dipped into the solution R and dried and subjected to FTIR analysis. On binding with $Cu^{2+}$ ions the -COOH band appeared with reduced intensity whereas the carbonyl group completely disappeared. The results showed that our method would be suitable to routinely analyse the $Cu^{2+}$ content in real samples.

KEYWORDS: Semi-arid soil, Rhodanine, Turn-off, $Cu^{2+}$ ions detection, Intensity enhancement

## INTRODUCTION

About 40% of land surface in the earth comprises of arid and semi-arid areas and the people mainly depend on agriculture for their day-to-day basic food. However, in regions like this agriculture is influenced by factors like high heat, limitation of water, bared soil, frequent drought, wind erosion and also has a vulnerable topography which is highly prone to natural hazards. Due to insufficient rainfall in these areas supplementary irrigation becomes necessary to keep agricultural production sustainable. Recycling of the available wastewater for agricultural irrigation becomes one of a commonly followed practice in arid and semi-arid regions. Soil salinity is yet another challenge commonly faced in the arid and semi-arid lands (Rao et al., 2013; Elkhatib & Moharem, 2015; Joshi, 2016; Abd-Alwahab et al., 2020).

High-intensity agriculture is a high-cost industry followed in modern days which leads to ground water contamination, greenhouse gas release, crop genetic diversity loss and eutrophication of lakes, streams, rivers and coastal marine ecosystems. Also, it is associated with the soil fertility loss, soil erosion, the increased incidence of livestock and crop diseases, and high chemical inputs. The ecosystem contamination leads to long term environmental implications and health problems (Steinberg & Hodge, 2018; Neaman et al., 2024).

In India, some parts of Western Rajasthan, Punjab, Haryana, and Gujarat consist of arid soil consisting of considerable quantity and number of soluble salts like gypsum, calcium carbonates and sodium salts. The Coimbatore district present in Tamil Nadu consists of semi-arid regions with red loamy soils. The soils of Erode district are mostly gravelly, stony, and sandy. Due to prevailing of a dry climate and because of absence of vegetation, the organic matter is also less. These have very infertile soil, but with appropriate application of fertilizers and irrigation, dry crops which are drought resistant and salt tolerant like barley, wheat, cotton, maize, millets, pulses, etc., can be grown (Sathyakumar & Sivakumar, 2007; Genova et al., 2022).

Generally arid soils are calcareous consisting of high quantity of free carbonates. Higher quantity of $CaCO_3$ which leads

to precipitation of copper as either carbonate or hydroxide. Contamination of copper due to copper pesticides leads to toxicity of organisms present in the soil. It is to be noted that a considerable number of these pesticides do not adhere strongly on the plant surfaces. Instead, they are leached from the surface of the leaves into the soil. A widespread issue arises because of the elevation in the copper levels in soils and for replanting high copper toxicity the in the soil becomes a significant challenge. This has prompted us to look into copper contamination in these agricultural backgrounds and solve these problems to facilitate replanting. Detection and quantification of $Cu^{2+}$ ions become important (Sathyakumar & Sivakumar, 2007; Rao *et al.*, 2013; Joshi, 2016; Steinberg & Hodge, 2018; Genova *et al.*, 2022; Neaman *et al.*, 2024).

We have synthesised sensor R having fluorescent properties, consisting a rhodanine and quinoline moieties (Vavoulidou *et al.*, 2005; Quang & Kim, 2010; Chen *et al.*, 2012; Kaur *et al.*, 2018) for sensitive and selective identification of $Cu^{2+}$ ions available in aqueous solutions. Sensor R was prepared by combining two raw materials which are very much available commercially. The probe exhibited characteristic properties like aggregation induced emission, fluorometric "turn-off" sensing mode towards the $Cu^{2+}$ ions, quick response and low detection limit (2.5 ppb). As a practical application Cu2+ ions detection by R adsorbed on a strip of test paper is also reported.

## MATERIALS AND METHODS

### General Methods

Analytical grade reagents and chemicals were procured from local commercial suppliers (Aldrich and Merck) and used as such without purification. A spectrum in the UV-Vis region was recorded using Jasco V-750 spectrophotometer. $^1$H NMR was measured on a Bruker Avance 400 MHz NMR Spectrometer using TMS as internal standard was used to evaluate chemical shifts. Fluorescence data was determined in a Horiba Fluoromax Plus Fluorescence spectrophotometer. FTIR spectra was recorded in a Bruker Alpha-II (ATR mode) instrument between 4000 and 400 cm$^{-1}$ region. DFT was carried out using Gaussian 9.0 software.

### Evaluation of Sensor Selectivity toward Cations by UV-Vis Spectroscopy

The sensor was evaluated with various cations by the addition of 2.0 mL of sensor solution to 2.0 mL of the cation solutions. After through mixing of the two solutions for 60 s, absorbance data was measured between 200 and 600 nm region with a scanning speed of 240 nm per minute against distilled water as a blank solution.

### Evaluation of Complex Sensor-$Cu^{2+}$ Stoichiometry

Aqueous solutions were separately prepared for the sensor R and the $Cu^{2+}$ ions. Both the solutions were appropriately mixed together in order to prepare a number of solutions having a range of mole fractions of $Cu^{2+}$ between 0 and 1, keeping the final concentration of the solution constant. Accordingly, volumes of 2.0, 1.8. 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, 0.2 and 0 mL of $Cu^{2+}$ solution were mixed with volumes of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 mL of the sensing solution maintaining the final volume of the solution as 2.0 mL After mixing for 60 s, the absorbance of mixed solutions at 308 nm was determined. Absorbances of the solution were normalized against equal volume of the blank solutions of cation in water.

### Titrations between $Cu^{2+}$ and R Solutions

Titration was done by the addition of 1.0 mL of 100 µM solution containing the sensor with 2.0 mL of $Cu^{2+}$ containing solutions of various concentrations. In this process the concentration of R at the end was 33 µM, and $Cu^{2+}$ solution concentration ranges from 0 to 133 µM. NMR spectra was recorded.

### Synthesis of R

3-a-carboxymethyl rhodanine (0.003 mol) and 2-chloro-6-methylquinoline-3-carbaldehyde (0.003 mol) were mixed and refluxed in presence of sodium acetate anhydrous (0.003 mol) dissolved in acetic acid glacial for 8 h. Resulted solution was cooled and the obtained precipitate was initially water washed, filtered and dried to yield the product.

$^1$H-NMR: solvent: DMSO-d$_6$ ppm($\delta$) 4.71 (1H, H-2'), 2.34 (1H, H-1''), 7.22-7.72 (4H, H-4'',5'',7'',8''), 8.27 (1H, H-6); $^{13}$C-NMR solvent: DMSO-d$_6$; ppm($\delta$) 20.80 (C-1'''), 45.36 (C-2'), 167.87 (C-1'), 167.29 (C-4), 196.79 (C-2), 115.74-160.87 (C=C carbons); Elemental Analysis: C, 50.70%; H, 2.91%; Cl, 9.36%; N, 7.28%; O, 12.67%; S, 16.93%.

### Determination of Copper Ions in Real Soil Samples

From Erode District in Tamil Nadu, soil samples of semi-arid in nature were collected and extracted using water and centrifuged to remove any solid particles. Then the pH of the solutions were adjusted to be between 4.0 and 6.5. The resulted solution was analysed by UV-spectrometry. Also, to validate the performance of our method the above solutions were added with known amounts of $Cu^{2+}$ (0.05 M) ions. The enriched samples were analysed with specrometry.

## RESULT AND DISCUSSION

### Synthesis of R

A one-step synthesis of a rhodanine sensor R bearing carboxy methyl rhodanine moiety and a quinoline moiety was reported to identify the presence of copper ions in water media (Figure 1a).

A mixture of 3-a-carboxymethyl rhodanine and 2-chloro-6-methylquinoline-3-carbaldehyde were refluxed for 8 hr in presence of anhydrous sodium acetate dissolved in glacial acetic acid to yield the product R (Figure 1b). It was characterised by NMR (Figures 2 & 8) and elemental analysis data.
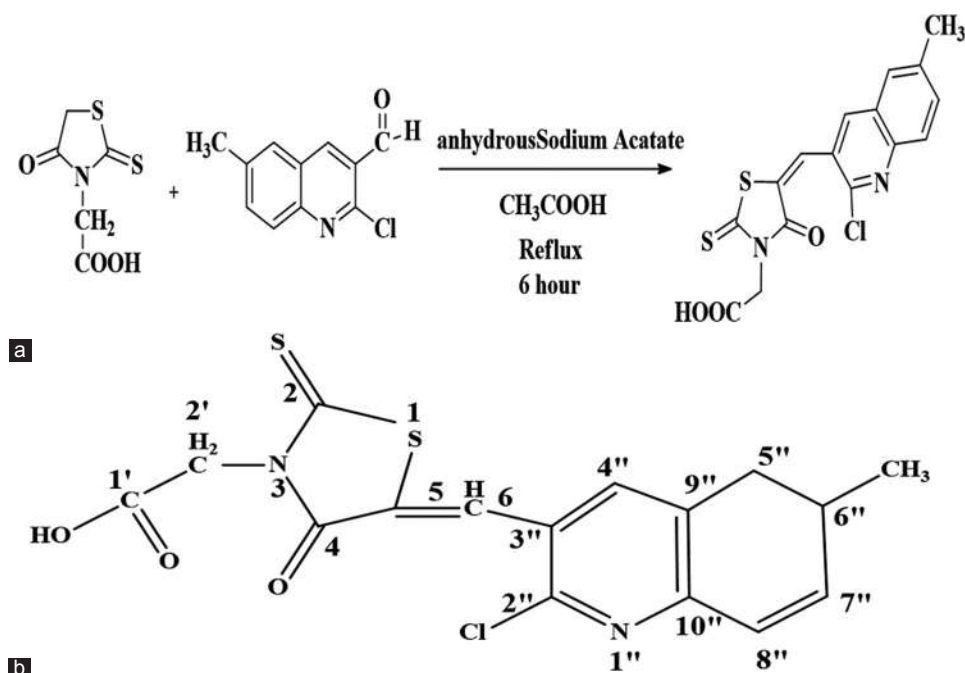
**Figure 1:** (a and b) Synthesis of the sensor R
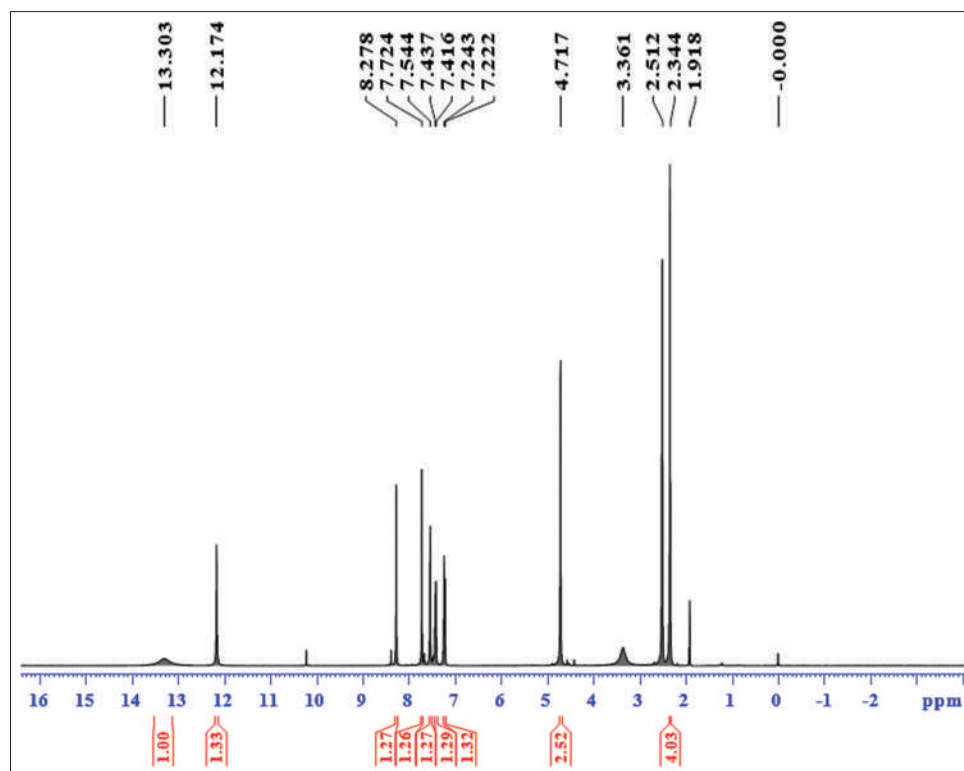


**Figure 2:** ¹H-NMR spectra of the Sensitizer R

In a rhodanine molecule, a UV spectrum at 308 nm typically corresponds to a significant absorption band, signifying the presence of a conjugated system within the molecule, most likely related to the thioamide group (C=S-N) which is responsible for the characteristic absorption in this wavelength range; this absorption band is often considered a key feature for identifying rhodanine compounds in UV-Vis spectroscopy.
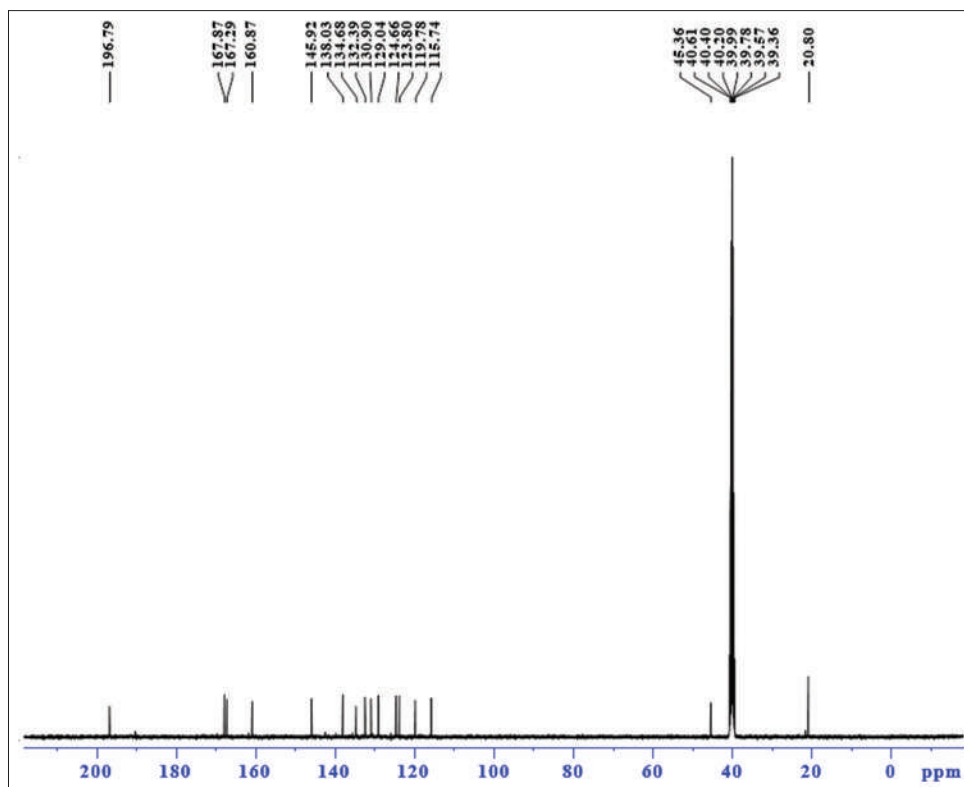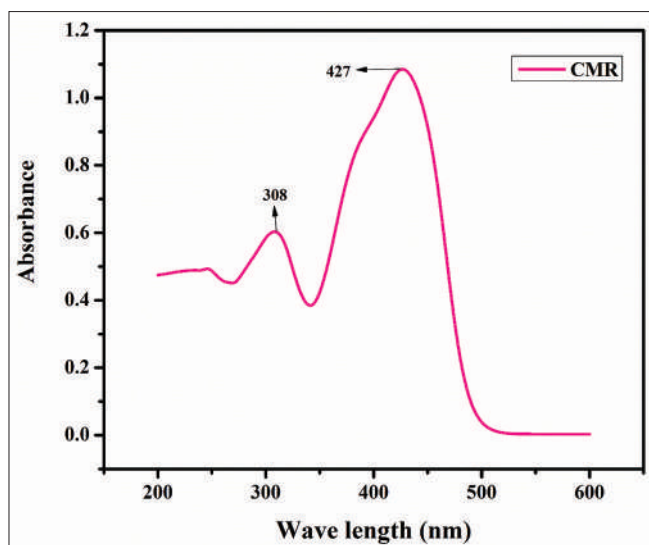
**Figure 3:** $^{13}$C-NMR spectra of the Sensitizer R



**Figure 4:** UV-Vis spectrum of sensor R



**Figure 5:** UV-Vis spectrum of sensor R in presence of other ions

## Photochromic Properties of Sensor

While developing a chemosensor one among the important parameter to be evaluated in the beginning is the selectivity of it towards a particular ion. This decides the sensing efficacy, thereby describes its capacity to identify certain metal ions in an unknown mixture. As we see the UV-Vis spectrum of sensor R in Figure 4, it exhibits a characteristic absorption band at 308 nm due to the presence of a thioamide group in the rhodanine moiety and at 427 nm due to the π-π transition of

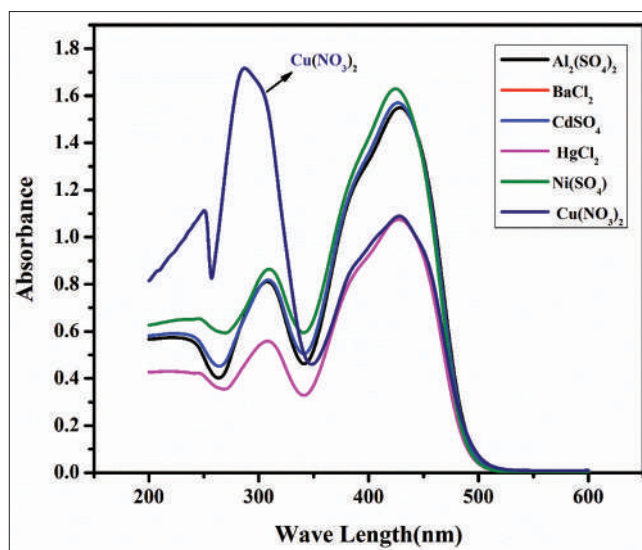the unsaturated carbonyl group extending into the quinoline moiety.

The selectivity of sensor R in presence of various positively charged ions was analysed using UV-Vis spectroscopy by carefully observing the changes taking place in the absorption bands of the spectra of the solution for sensor R (10.0 µM) along with other ions. Upon addition of equal volume of cationic solutions like $Al^{3+}$, bivalent ions like $Cd^{2+,}$ $Ni^{2+}$, $Hg^{2+}$, $Ba^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Mg^{2+}$, $Ca^{2+}$, $Fe^{2+}$ and $Sn^{2+}$ and

monovalent ions such as Li+, Na+ and K+, in to the solution of R, only a marginal change in the intensity has happened in the absorption band at 308 nm was noticed. But whereas a 100% enhancement in intensity is observed for $Cu^{2+}$ ions (Figure 5).

Equal volumes of copper cation solutions of various concentrations 100, 80, 60, 40 and 20 μM. were added separately in to the solution R and the UV-Visible spectrum was recorded. The intensity of the absorption band at 308 nm of various cations in presence of sensor R were presented in Figure 6. It could be observed from the figure 6 that, the intensity at 308 nm of sensor R solution enhanced quantitatively after the addition of $Cu^{2+}$ ions, and remains unchanged in the intensity for more than 1 hr. An excellent selectivity of R for $Cu^{2+}$ cation was demonstrated through these results by UV-Vis spectroscopy. The intensity of the absorption band at 427 nm remained the same. The pH was maintained between 4.0 and 6.5 for all the solutions during the analysis.

## Determination of Binding Mode between Sensor R and $Cu^{2+}$

In order to further evaluate the sensor properties of the rhodamine derivative R with $Cu^{2+}$ the binding mode between them was determined by continuous-variation method. The Job's plot was built by varying the molar fraction of $Cu^{2+}$ ion from 0 to 1, maintaining 0.2 mM as the final concentration of the solution and the absorption value was measured. From the obtained Job's plot, the binding stoichiometry was determined for the complex formed between $Cu^{2+}$ ion and the sensor R which corresponds to the peak value of absorbance on the x-axis. The peak value of absorbance could be noted at a mole fraction of 0.38, suggesting a stoichiometry of 2:1 for the complex obtained between sensor R and $Cu^{2+}$.
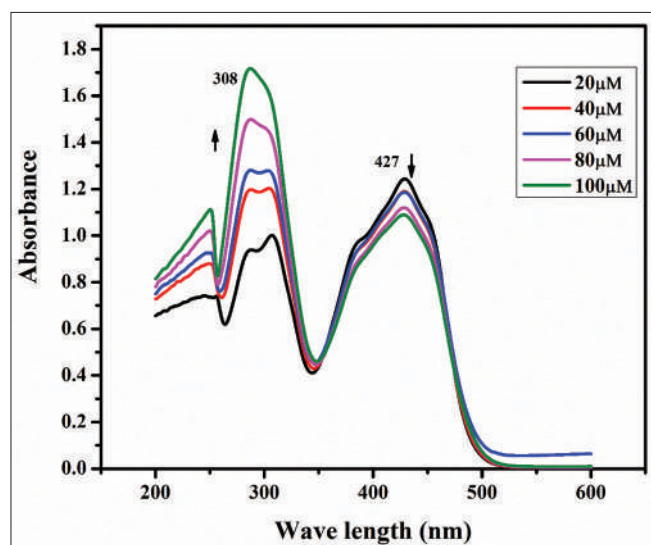


**Figure 6:** Spectrum in the UV-Vis range of sensor R in presence of copper cation solutions of various concentrations 100, 80, 60, 40 and 20 μM
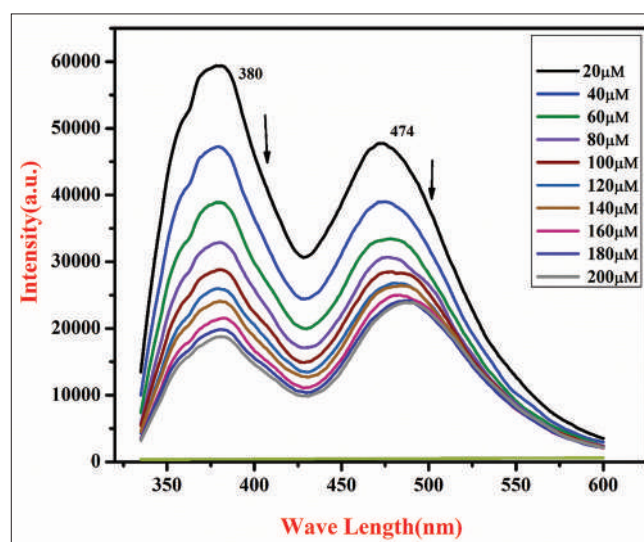
## Fluorescence Spectral Characteristics

The behavior of sensing of $Cu^{2+}$ ion by R was also studied by the measurement (Figure 7) of fluorescence behavior in DMSO-water (4:6, v/v) upon excitation at 308 nm and maintained at a PH=7.0 by buffering it with HEPES solution. Around 380 nm an emission band was noticed upon the addition of copper ions ($20$-$100 \times 10^{-3}$ μM) in small increments, and the fluorescence intensity decreased gradually with the $Cu^{2+}$ concentration increasing indicating the formation of a complex. Due to the complex formation the chemo sensor would have exhibited a very efficient fluorescence response.

The sensing mechanism was supported by NMR analysis (Figures 2 & 8). The spectral data showed that, upon treatment of $Cu^{2+}$ ions with the sensor solution R, all the signals had showed an up-field shift to a smaller extent. But the methylene protons signal at 4.79 ppm disappeared and shifted up field to appear as a broad peak at 3.62 ppm. This result proved that $Cu^{2+}$ ion was attached to the methylene protons. Methylene protons being acidic in nature due to the more electronegative groups on the either side. Since this is nearer to the thioamide group of the rhodamine, the intensity gets enhanced.

Equal quantity of 10.0 mL each of 10 mM $Cu^{2+}$ solution (10 mM) in distilled water and 10 mM solution of the sensor R in DMSO/ethanol (v/v=2/8) were taken from their stock solution. Filter paper strips were dipped into the solution R and dried. The dried filter paper coated with R was once again dipped in the 10 mM of $Cu^{2+}$ solution and once again dried in an oven. The dried filter paper was subjected to FTIR analysis.

The broad absorption band observed at 3300 cm⁻¹ of the IR spectrum of the sensor R is assigned to hydroxyl group of the acid moiety and absorption band at 1650 cm⁻¹ is due to carbonyl carbon. On binding with $Cu^{2+}$ ions the -COOH band appeared with reduced intensity whereas the carbonyl group completely



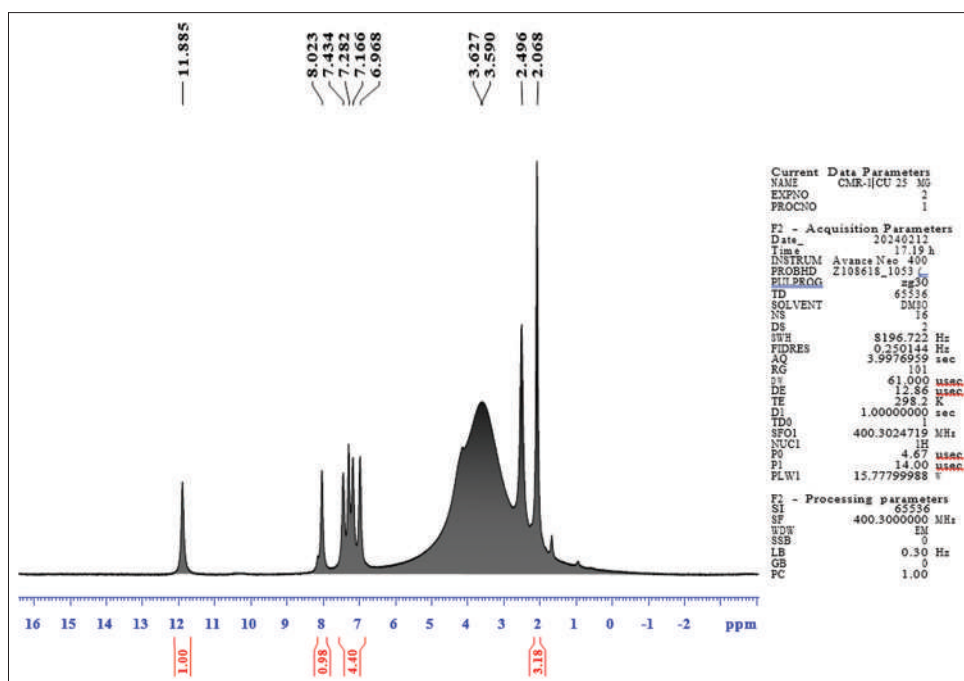**Figure 7:** Fluorescence Spectral Characteristics of R with copper ions

**Figure 8:** $^1$H-NMR spectra of the compound S with $Cu^{2+}$ ion



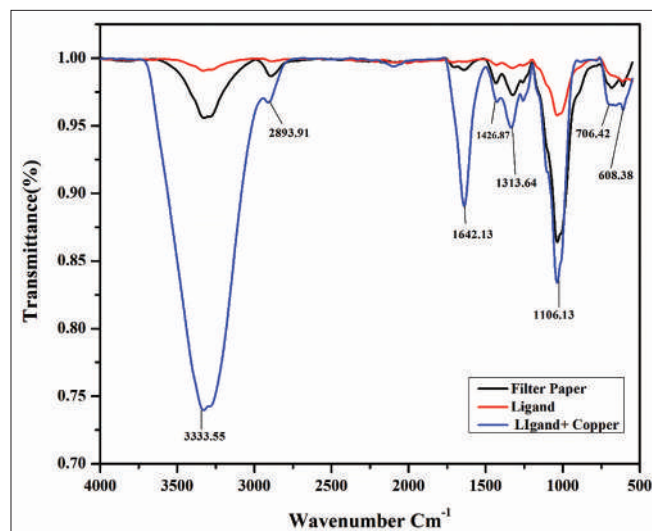**Figure 9:** FTIR spectra of the compound Filter paper, R and R with $Cu^{2+}$ ion



**Figure 10:** FTIR spectra of the Filter paper, R and R with various concentration $Cu^{2+}$ ion

disappeared indicating that these two groups are involved in the complex formation (Figures 9 & 10).

## Determination of $Cu^{2+}$ in Real Water Samples

The soil samples of semi-arid in nature available in the Erode District in Tamil Nadu were collected and extracted using water and centrifuged to remove any solid particles. Then the pH of the solutions were adjusted to be between 4.0 and 6.5. The resulted solution was analysed by UV-spectrometry. Also, to validate the performance of our method the above solutions were added with known amounts of $Cu^{2+}$ (0.05 M) ions. The enriched samples were analysed with spectrometry and the
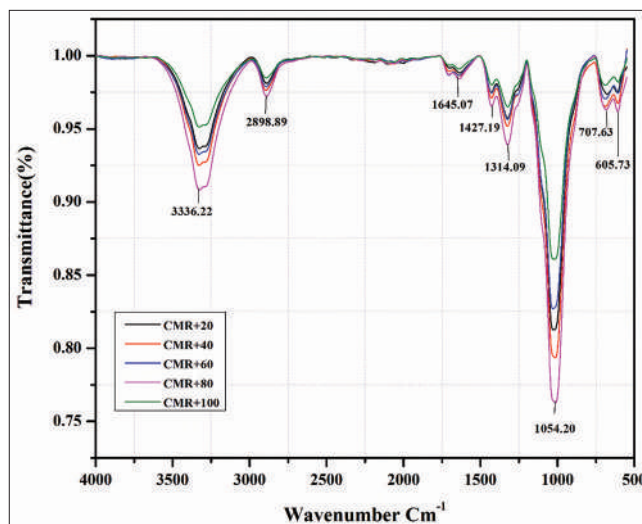
results are shown in the Figure 8. The results as exhibited in the Figure 8 showed a good correlation between the determined and theoretical concentration, indicating that the method performance was adequate and could be used for determination of $Cu^{2+}$ cation in real semi-arid soil samples.

## Density Functional Theory Studies

The theoretical calculations were done with the Gaussian 9.0 software to get the optimised structure of CMR and CMR-$Cu^{2+}$. Electronic calculations were performed using the B3LYP (Becke-3 Parameter-Lee-Yang- Parr) functional and the LANL2DZ (Los Alamos National Laboratory 2 Double-Zeta)
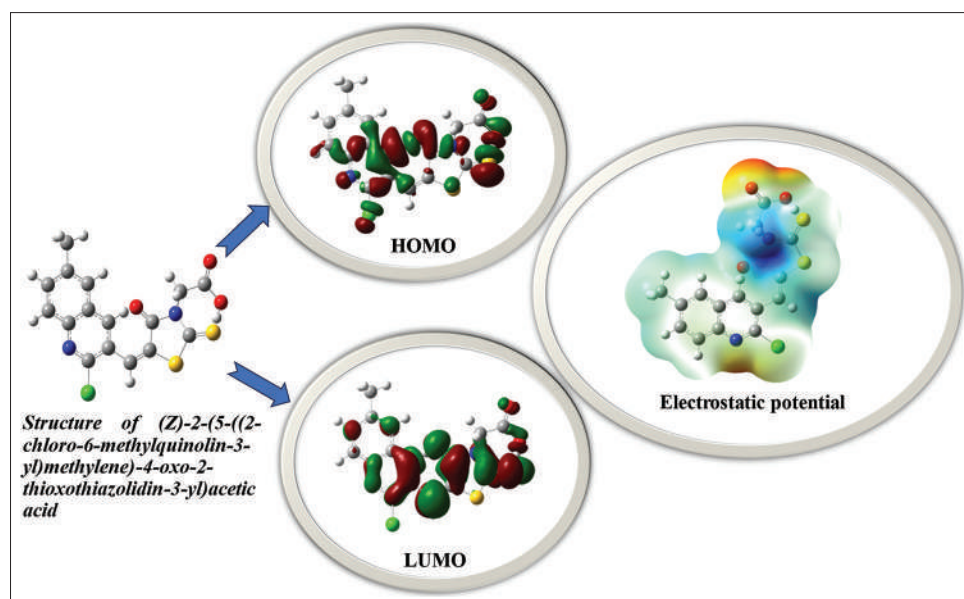
**Figure 11:** Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO)

basis set for the copper (Cu) atom with the 6-311++G(d, P) basic set for carbon (C), hydrogen (H), nitrogen (N), sulphur (S), chlorine (Cl) and oxygen (O) atoms. Frontier molecular orbital analysis was also undertaken to study about the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO)) in order to determine the band gaps of the proposed complex (Figure 11).

## CONCLUSION

Even though copper ions could be detected with various types of fluorophores like rhodamine, coumarin, fluorescein and anthracene by using techniques like absorption spectroscopy (AAS), emission spectroscopy (AES) and inductively coupled plasma mass spectroscopy (ICP-MS). But getting access and tested for this by the farmers is very difficult (Jung *et al.*, 2009; Li *et al.*, 2014; Cotruvo Jr. *et al.*, 2015; Sfrazzetto *et al.*, 2016; Udhayakumari *et al.*, 2017; Wu *et al.*, 2017). To facilitate in this aspect, in the present study we have reported the use of sensor R bearing carboxy methyl rhodamine moiety and a quinoline moiety to detect copper ions in aqueous media. The obtained probe, due to water-insolubility nature (forming aggregates), exhibits strong green emission and irreversible electrode reaction. When interacting with copper (II) ions, the probe demonstrates "turn off" sensing event with irreversible chemical and reversible electrode reactions with cathodic potential shift. Besides, filter test papers coated with the probe were applied for IR spectra adder dipping in the water solution for detection of copper (II) ions. Detection of coper using simple spectrophotometric methods using UV-Vis, IR and fluorescence phenomenon is in high demand because of its low cost, easy operation, and simplicity.

## ACKNOWLEDGEMENT

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