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Paper based field deployable sensor for naked eye monitoring of copper (II) ions; elucidation of binding mechanism by DFT studies

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ABSTRACT

The study demonstrates the fabrication of test strips made from newly synthesized ortho-Vanillin based colorimetric chemosensor (probe **P**) that could be employed as field deployable tool for rapid and naked eye detection of Cu^{2+}. Upon addition of Cu^{2+} to the chemosensor, it exhibits rapid pink color from colorless and can be easily seen through the naked eye. This probe exhibits a remarkable colorimetric "ON" response and the absorbance intensity of the probe enhances significantly in presence of Cu^{2+}. The sensing mechanism has been deduced using FTIR, XPS, LCMS and DFT studies. The binding mechanism of the probe to Cu^{2+} was substantiated by DFT studies. HOMO of the probe suggests that a high electronic density resides on O, N atoms and thus these are the favorable binding site for the metal ions. Study revealed that the **P** + Cu^{2+} complex is −35.64 eV more stable than individual reactants. The Cu^{2+} binds to the probe in 1:1 stoichiometry with a binding constant of $2.6 \times 10^4\ M^{-1}$ as calculated by Job's plot and Benesi-Hildebrand plot. The chemosensor shows 1.8×10^{-8} M detection limit, which is considerably lesser than that of the WHO admissible limit of [Cu^{2+}] in drinking water. Possible interfering ions namely Ca^{2+}, Mg^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Mn^{2+}, Al^{3+} and Cr^{3+} do not show any appreciable interference in the colorimetric response of the probe towards Cu^{2+}. Particularly, the colorimetric "ON-OFF-ON" responses are proved to be repeated over 5 times by the sequential inclusion of Cu^{2+} and S^{2-}. Sensitivity of the probe in real-time water and blood samples is found at par with results with AAS and ICP-OES techniques. Further, the reversibility of the probe and the easy fabrication of deployable strips for real-field naked eye detection of Cu^{2+} suggest importance of synthesized probe.

1. Introduction

Among transition elements, copper is an essential and third most abundance among the required trace elements in the human body. It plays a critical role as a catalytic cofactor for a variety of metalloenzymes involved in transcriptional events [1] and other important fundamental physiological processes in various organisms [2]. However contamination of copper and its strong toxic effects on biological community are a threat, due to its wide use in domestic, agricultural and industrial practices [3] Changes in the cellular balance of copper ion leads to serious disorders associated with neurodegenerative diseases like Wilsons disease [4], Menkes disease [5], Alzheimer's disease [6], Prion disease and familial amyotrophic lateral sclerosis [7]. The World Health Organization (WHO) has set the maximum granted limit of copper in drinking water at 2 mgL^{-1} (31 μM) [8]. Thus, event of authentic, easy and swift process for examining the copper quantity in environmental and biological samples is necessary. Numerous spectroscopic techniques [9] and electrochemical techniques [10] are implemented for Cu^{2+} estimation but they usually are time consuming, expensive and complicated. Optical spectroscopic techniques with fluorescent/non-fluorescent receptors have captivated researches attention with advantages like quick, sensitive and non-destructive study, low cost and prompt detection of metal ions by a simple enhancement in absorbance responses, the development and designing of colorimetric chemosensors for detection of biologically and environmentally important transition metal ions have received considerable importance [11]. Metal chalcogenide/quantum dots (QDs) have been reported as fluorescence based sensors/receptors with its excellent stability and photophysical properties [12]. Due to their tunable optical properties [12a,13], it has gained importance in numerous fields such as photovoltaics [14], bioimaging [15] and as sensors [16,17]. But unlike chemosensors it requires sophisticated instruments such as Spectrofluorophotometer and fluorescence microscope with skilled labor for sensing and can't be used as naked eye sensors based on color change. A number of receptors have been reported for the determination of Cu^{2+} ions [18]. However, most of the recently delineated Cu^{2+} sensors are quenching types [18e,19]. Characteristics like the interference of coexisting metal ions [18g,20], the convenient probability and the high response time still limit the practical application of Cu^{2+} chemosensors. Number of chemosensors

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has been developed for the consideration of more sensitive detection [21] and for their improvement with respect to water solubility. However reports on water-soluble chemosensors that can be employed in aqueous media do exist [18g,22]. Nevertheless, still there is a great scope and demand for novel and productive chemosensors for Cu^{2+}, particularly for those chemosensors that can function with high selectivity and sensitivity in aqueous media [23].

Colorimetric sensors for the detection of Cu^{2+} have some drawbacks like high response time [18g,24], poor sensitivity [1,18f,25], interference of other metal ions [26]. A bis-triazole-attached azobenzene chemosensor for selective sensing of Cu^{2+} was reported by Kannan et al. nonetheless the synthetic procedure of the same was a tiresome one [27], Sheng et al. detailed a test kit for colorimetric detection of Cu^{2+} with a detection limit of 1.2μM [25]. Lu et al. reported a gold nanoparticle based sensor for selective and sensitive detection of Cu^{2+} with high response time but had Co^{2+} interference [28]. Despite their assertion of Cu^{2+} sensing in aqueous medium and still require H_2O/DMSO (60/40; v/v) solvent mixture. Gunnlaugssaon et al. reported an azobenzene chemosensor for naked-eye detection of Cu^{2+} but Cd^{2+} and Zn^{2+} modulated the absorption spectrum of other metal ions at greater concentrations than that of Cu^{2+} [29]. Similarly, numerous of chemosensors described in the literature for detection of Cu^{2+} are on the basis of detection of multiple ion phenomenon [11b–d,30]. The chemosensors with multiple ion sensing are not demonstrated to be effective ones as there are possibilities of interference in the detection of targeted metal ion. Progress of economic, ion selective and naked eye detectable chemosensors are strongly in demand.

Rhodamine derivative based chemosensors, because of their excellent photo physical properties have attracted the attention of researchers. The spirolactam ring opening accords to a large molar extinction coefficient, absorption and emission at higher wavelengths, light stability etc. [11e]. Furthermore, the symmetry linking the nonfluorescent/noncolorimetric form and the highly fluorescent/colorimetric open-ring form anticipates a promising strategy to develop "turn-On" sensors. Hence, Rhodamine derivatives are extensively used as a colorimetric/fluorescent probe for the detection of various metal ions such as Hg^{2+} [11f], Fe^{3+} [11g], Zn^{2+} [31], Cr^{3+} [32], Pb^{2+} [33], Au^{3+} [34], Al^{3+} [35].

Exploring the chemosensors with above mentioned merits, herein we introduce ortho-Vanillin moiety to Rhodamine (probe **P**) which is highly selective and gives quick response to Cu^{2+} ion (Scheme 1). To the best of our knowledge, the synthesized probe is the first example of o-Vanillin based Rhodamine derivative as Cu^{2+} sensing probe. Our probe exhibits high selectivity and sensitivity compared to the recently developed Cu^{2+} sensors [36]. Use of immobilized probe strips for on-field naked eye detection, for real time industrial waste water analysis and for blood sample analysis shows its novelty and possible real time applications.

2. Experimental

2.1. Materials and methods

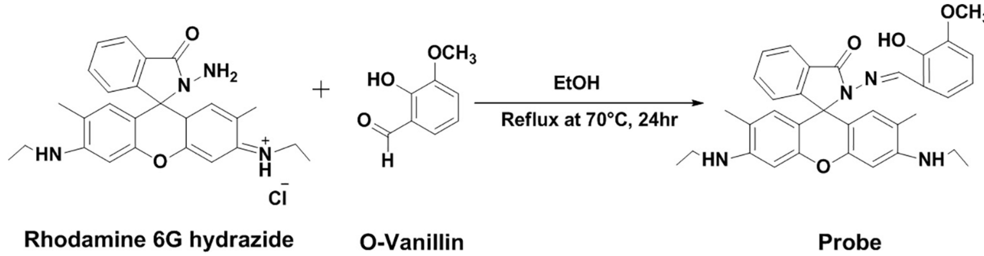
All the solvents used were of analytical grade and used without further purification, unless stated. Solvents were dried according to the standard procedures. Metals used are nitrates of Hg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, Ag^{+}, and chloride salts of Cr^{3+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Pb^{2+} and Cu^{2+} were commercially procured from Sigma-Aldrich Chemicals Co., and employed without any further purification. Throughout all the experiments double distilled water was used. All reactions were magnetically stirred and thin-layer chromatography (TLC) by Merck TLC Silica gel 60 F_{254} coated plates were used to monitor the reaction. A JASCO-460 plus FT-IR spectrophotometer was utilized to carry out the FT-IR spectra of the samples as pressed KBr pellets. AAS measurements were carried out using Agilent 55B AA spectrometer. ICP-OES measurements were recorded Agilent 5100 VDV ICP-OES instrument. NMR spectroscopic measurements in $CDCl_3$ were recorded with an Agilent spectrometer 400. The ^1H NMR and ^{13}C NMR chemical shift values are represented in ppm (δ) correlative to $CDCl_3$. Waters SynaptG2 LC-MS spectrometer was used to record the Mass spectra. Absorbance (UV–Vis) spectroscopic study was carried out with a Shimadzu UV-1800 spectrophotometer using quartz cuvette. X-ray Photoelectron Spectroscopy (XPS) analysis was performed with an Esca Pkus Oxford XPS instrument.

2.2. Synthesis of probe

Rhodamine 6G Hydrazide was synthesized as per the reported procedure [18a], (see supporting information Figs. S1–S3). In 30 mL of absolute ethanol, Rhodamine 6G Hydrazide (0.48 g, 1 mmol) and ortho-Vanillin (0.28 g, 1.86 mmol) were dissolved, and the solution mixture was refluxed at 70 °C for 24 h. The obtained compound was filtered and the residue was washed several times with absolute ethanol to remove any unreacted starting material. The purity of product was checked by spotting a product on chromatographed silica plate [60 F_{254}] with hexane/ethyl acetate (7:3, v/v) as eluent to get a light pink solid (probe **P**) in 85% yield. mp = 256 °C. ^1H NMR shows ($CDCl_3$, 400 MHz) δ (ppm): 10.93 (1H, s, –CHO), 9.11 (1H, s, CH=N), 7.96–7.98 (3H, Ar—H), 7.46–7.48 (3H, Ar—H), 7.24 (1H, Benzene-H), 7.05–7.05 (1H, d, Benzene-H), 6.78 (1H, t, Benzene-H), 6.70 (1H, d, Ben—H), 6.38 (2H, s, xanthene-H), 6.30 (2H, s, xanthene-H), 3.47–3.80 (2H, $NHCH_2CH_3$), 3.20 (6H, q, $NHCH_2CH_3$), 1.85 (6H, s, xanthene-CH_3), 1.30 (t, 6H, $NHCH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm): 14.7, 16.7, 38.2, 55.9, 66.2, 76.7, 77.0, 77.3, 96.9, 105.3, 113.2, 118.0, 118.4, 118.5, 123.2, 123.3, 123.9, 127.6, 128.3, 129.0, 133.5, 147.5, 148.1, 148.2, 151.5, 151.7, 152.7 and 164.5. ESI-MS, calculated for $C_{34}H_{39}O_3N_4$ *m/z* = 562.65, Found: 563.16 (see supporting information Figs. S4–S5).

2.3. Synthesis of Cu^{2+} complex of probe

A 10 mL methanol solution of $CuCl_2$ (1 mmol) was added slowly to a magnetically stirred solution of probe (1 mmol) in CH_3CN. The mixture was stirred for 10 min; a pink colored solution was obtained and left for slow evaporation, washed several times with distilled water and dried over vacuum. The corresponding IR and mass spectrum of the complex have been given in electronic supplementary information (Figs. S5 & S9). Spectroscopic characterization data for the complex: IR (ν, cm^{-1}): 3320, 1607, 1498, 1444, 1372, 1302, 1182, 1126, 1081 and 1015. ESI-



Scheme 1. Synthesis scheme for the probe.

MS: *m/z*. Calculated for **P** + Cu^{2+} complex = 624.06 and found = 623.91.

2.4. UV–Vis spectral studies

A stock solution of **P** (2.0×10^{-5} M) was prepared in CH_3CN. Respective cations (1.0×10^{-3} M) solutions were prepared in deionized water. For titration experiments, 2 mL (10 μM) of probe solution was used in a quartz optical cell with an optical path length of 10 mm; Micropipette was used to add the metal ion stock and allowed to equilibrate the solution. After the addition of ions spectral data were recorded at 1 min. By adding appropriate amount of the anions/cations stock solution into 2 mL (10 μM) probe solution, test samples were prepared for selectivity experiments. Reversibility study was done using 1.0×10^{-3} M solution of S^{2-} (aq.).

2.5. Binding constants and stoichiometry calculation

The formation of the binding constant for respective complexes was evaluated using Benesi-Hildebrand (B—H) plot (Eq. (a)) [37].

$$1/(A - A_{ini}) = 1/K_a\,(A_{max} - A_{ini})\,[M^{n+}] + 1/(A_{max} - A_{ini}) \quad \text{(a)}$$

Here A_{ini}, A_{max} and A represents the absorption potency of free probe, the maximal absorption intensity were pronounced at 523 nm at a certain concentration of the metal ion added. Binding stoichiometry for the formation of complex was also confirmed by Job's plot.

2.6. Limit of detection

On the basis of the UV–Vis titration, limit of detection was calculated (Fig. 5b). The absorption spectrum of the probe was measured 25 times and the standard deviation of blank measurements was achieved. Plot of absorption intensity at 523 nm vs concentration of Cu^{2+} is used to calculate the slope. The limit of detection was deliberated with the following equation [38].

$$DL = (3 \times S_1)/S \quad \text{(b)}$$

where DL (limit of detection), S_1 is the standard deviation of a regression line and S is the slope.

2.7. Fabrication of testing strips

Nitrocellulose strips were washed with distilled water followed by coating with poly vinyl alcohol (PVA) and dried. The dried strips were coated with probe and finally dried at room temperature. These strips were used for naked eye detection of Cu^{2+}.

2.8. Computational studies

Gaussian 09 programs Becke's three parameterized Lee-Yang-Par (B3LYP) exchange functional with 6-31G* basis sets were used to study the density functional theory (DFT) calculations for the optimization of the **P** and the and **P** + Cu^{2+} complex.

2.9. Detection of Cu^{2+} in industry waste water and blood samples

Industrial waste water was collected from paper and plastic industry from Harohalli industrial area, Bangalore rural, Karnataka, India. Human whole blood samples were collected and samples were digested as per the established standard protocol [39]. Details of the certified reference material are attached in Fig. S14.

3. Results and discussions

3.1. Designing of probe

The design of probe makes it a suitable candidate to function as an intramolecular charge transfer (ICT) probe. The xanthene part is expected to act as electron acceptor while the remaining part is expected to act as electron donor (Fig. 1). The effectiveness as an ICT probe was reproduced in terms of big perturbation in the absorption pattern as well as visual appearance (from colorless to pink) of the probe upon addition of Cu^{2+}. A strong intramolecular hydrogen bonding is quite likely between the –OH and imine —N which helps in blocking the possibility of anionic analytes interaction (Scheme 1).

3.2. Visible detection of Cu^{2+}

Each metal ions like Cr^{2+}, Ag^{+}, Cd^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Ca^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} were added separately to probe in 10 μM solution. This displayed a recognizable color change from colorless to pink only for Cu^{2+} in CH_3CN, whereas other metal ions did not show any color change (Fig. 2). For this reason in case of Cu^{2+} easily observable color change with probe can be used for "naked eye" detection of Cu^{2+}. The emergence of the color change from colorless to pink was regarded to modulate the intramolecular charge transfer of probe upon effective coordination of Cu^{2+} through nitrogen atoms along with hydroxyl group of the probe.

3.3. Effect of probe concentration, nature of the target analyte based on various anions and response time of the probe

The effect of probe concentration used for Cu^{2+} analysis was also investigated. Upon the addition of increasing amount of Cu^{2+} it looked like the concentration of probe was proportional to the absorbance maximum. But, within the dynamic range, the probe concentration did not play significant role in sensing of Cu^{2+}. In the following tests, the concentration of probe used was fixed to 10 μM. According to the obtained results, the optimized condition selected for Cu^{2+} analysis was: 10 μM in CH_3CN (Fig. S6a). The ability of the probe to detect Cu^{2+} present in various salt forms namely as $CuCl_2$, $Cu(SO)_4$ and $Cu(NO_3)_2$ is investigated. The graph in Fig. S6b, shows more or less, a linear increase in the absorbance with concentration, for various analyte forms. The probe complex with copper, of $CuCl_2$ has shown better sensitivity, compared to $Cu(NO_3)_2$ and $CuSO_4$. The structure of anions could hence influence the sensitivity of the probe. The probe when complexed with $CuCl_2$ shows a highly-linear curve indicating the accuracy of the

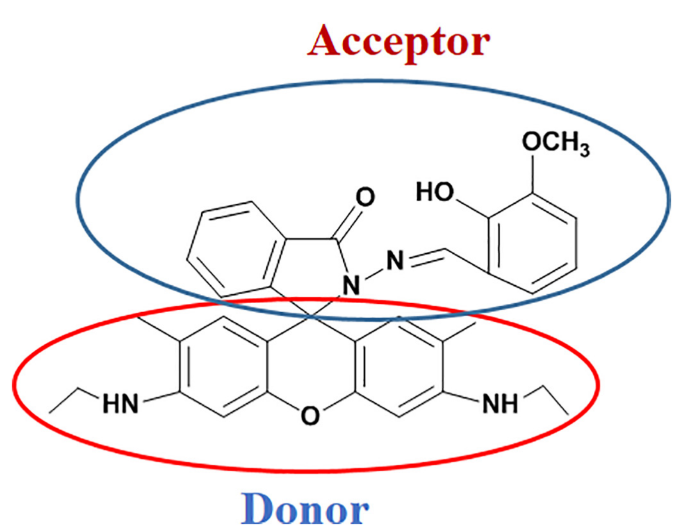


Fig. 1. Representation of donor and acceptor part of probe.

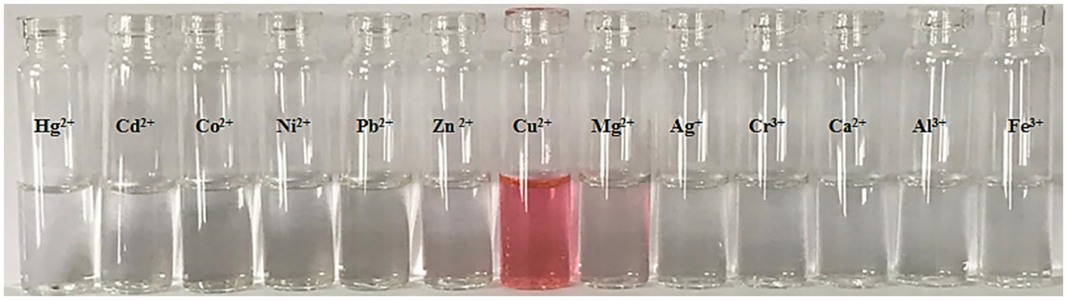


Fig. 2. Digital photograph of colorimetric changes of probe upon addition of various metal ions as their chloride salt.

results that would be obtained when Cu^{2+} is detected in $CuCl_2$ salt forms. Favorable effect of anions on the probe complex follow the order $Cl^- > NO^{3-} > SO_4^{2-}$, which is in accordance with distribution coefficient ($Log_{10}Kd$) values. The response time of probe with Cu^{2+} was studied to examine the sensitivity of probe towards Cu^{2+}. As shown in Fig. S6c, the absorbance of probe at 527 nm rapidly increased with the addition of Cu^{2+} (10 μM), reached maximum at 60 s and stabilized. These results indicate this probe is a sensitive chemosensor for the detection of Cu^{2+}.

3.4. Effect of pH

To investigate the influence of different acid-base concentration on the probe and to obtain a suitable pH in which probe can efficiently detect Cu^{2+}, the titration experiments were performed at various pH conditions ranging from 3 to 9. The probe by itself appeared pink at a pH below 3 i.e.; the probe lost its stability (was completely protonated) leading to spirolactam ring opening. The probe remains stable beyond pH 3. However, the sensitivity of the probe was highest at pH 6 which is attributed to opening of spirolactam ring as observed from Fig. S7. The formation of $Cu(OH)_2$ beyond pH 6 reduces the binding affinity and coordination tendency. Hence, pH 6 was considered as better operating pH.

3.5. Selectivity

Selectivity of probe towards Cu^{2+} among a wide range of biologically and environmentally important metal ions including Cr^{2+}, Ag^+, Cd^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Ca^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} were investigated. Fig. 3 shows the absorption response of probe (10 μM CH_3CN) solution towards a series of the above mentioned metal ions. There was barely any change in the absorption spectra of the probe was observed but, under similar conditions, as we expected the probe was colorless and non-fluorescent due to the existence of nonconjugated spirolactam structural form of probe as shown in Scheme 1. The instant color change in presence of Cu^{2+} suggests that probe could serve as a "naked-eye" chemosensor for Cu^{2+} (Fig. 3 inset).

3.6. UV–Visible studies

With probe all spectroscopic measurements were performed in its 10 μM CH_3CN solution. The reaction of probe with Cu^{2+} was studied by UV–Vis titration. In the visible region the probe by itself showed almost nil absorption. The colorless probe solution turns pink and a new strong absorption band appears at 523 nm with increase in concentration of Cu^{2+} ions (Fig. 4). The "switch-On" behavior for the intense absorption peak at 523 nm suggests spirolactam ring opening in probe on Cu^{2+} coordination [40]. The isosbestic points at 255, 290 and 330 nm designates the formation of a single species between the probe and the Cu^{2+} (Fig. S8). The absorbance at 523 nm increases with Cu^{2+} concentration and saturates at ~250 $\mu molL^{-1}$ of Cu^{2+}. There is no significant change in the absorbance when the concentration Cu^{2+} increases from 250 to 350 $\mu molL^{-1}$. Changes in the ultraviolet-visible (UV–Vis) absorption spectrum and the color change could be accompanied by the ligand-to metal charge transfer transition (Fig. 4). Absorbance of Cu^{2+} in CH_3CN was recorded (Fig. 4 inset) to rule out its effect on the absorbance of the probe.

The binding affinity of the Cu^{2+} towards the **probe** was evaluated from the spectrophotometric titration experiment using Benesi-Hildebrand plot and the binding constant (K_a) is $2.6 \times 10^4\ M^{-1}$ and the correlation coefficient (R^2) is 0.9829 (Fig. 5a). The detection limit of Cu^{2+} using probe is 1.8×10^{-8} M (Fig. 5b).

Job's plot results confirmed 1:1 binding stoichiometry for binding of Cu^{2+} and probe (Fig. 6a) which is further corroborated by mass spectra (Fig. S5b). Competitive binding of the probe to Cu^{2+} were determined in presence of other metal ions (500 $\mu molL^{-1}$) like Hg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, Ag^+, Cr^{3+}, Ca^{2+}, Al^{3+}, Fe^{3+} and Pb^{2+} (Figs. 2, 6b). The UV–Vis spectral study confirms that probe selectively binds to Cu^{2+} in the presence of different metal ions. Competitive absorption response suggests that there is no significant interference by other metal ions and the probe selectively binds to the Cu^{2+}. This results further support that probe could serve as a sensitive naked eye chemosensor for Cu^{2+} detection.

3.7. Selective response of P + Cu^{2+} media to various interfering anions

From the UV–Vis spectroscopic studies, we infer that probe selectively binds with Cu^{2+} to form **P** + Cu^{2+} complex with substantial change in its spectral behavior. One of the important features of the chemosensor is its elevated selectivity as well as the reversibility in

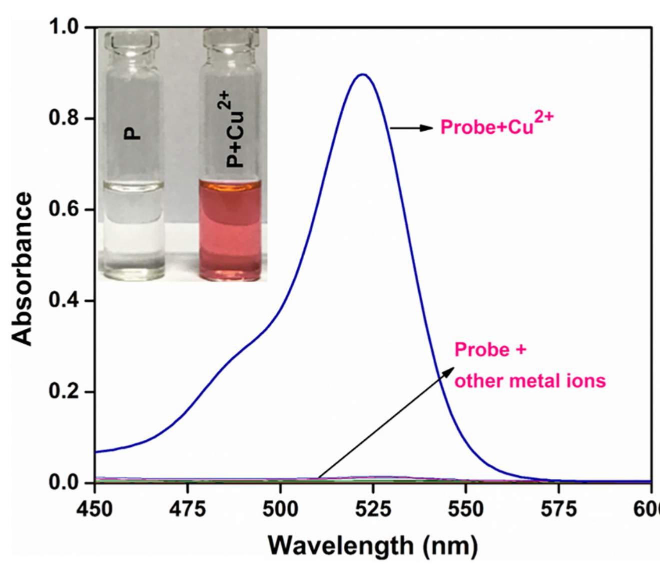


Fig. 3. Selectivity absorption changes of P upon addition various ions. Inset: L to R: digital photographs of P and P + Cu^{2+}.

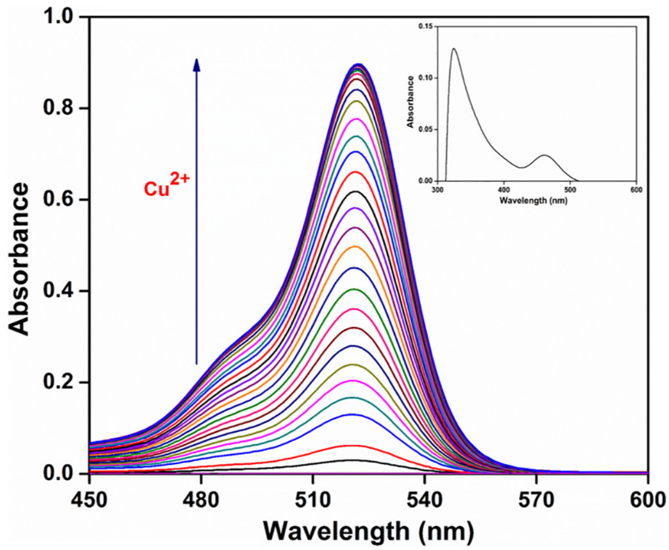


Fig. 4. UV–Vis titration spectra of probe with increasing Cu^{2+} ion concentration Inset: Absorbance of Cu^{2+} in CH_3CN (without the probe).

the complexation of any probe to be used as a chemical sensor for the determination of specific metal ions. Accordingly, we have measured the impact of different anions on the reversibility of **P** + Cu^{2+} complex to regenerate the probe. The probe could be revived by the addition of S^{2-} to the **P** + Cu^{2+} solution mixture (Figs. 7a, S12). Binding reversibility of Cu^{2+} to probe was also established in the presence of aq. Na_2S (500 μmolL^{-1}) through UV–Vis spectral studies. Addition of S^{2-} to the **P** + Cu^{2+} complex leads to the reverse changes in the absorption spectra (Fig. S12). Additionally, for better understanding, we carried out UV–Vis titration for this complex. Upon addition of the S^{2-} (aq.) to the **P** + Cu^{2+} complex deep pink solution of **P** + Cu^{2+} complex at 523 nm absorption band disappears, as well as the solution become colorless (Fig. 7a, inset). The concentration of $S^{2-} > 7.91 \times 10^{-7}$ M alters the absorbance of **P** + Cu^{2+} which is calculated using LOD calculation (Fig. 7c). The dissociation constant of Cu^{2+} from **P** + Cu^{2+} complex upon addition of S^{2-} (which is calculated by change in absorbance of **P** + Cu^{2+} upon addition of S^{2-}) is 1.06×10^5 M^{-1} and a correlation coefficient (R^2) is 0.9887 as evaluated from the spectrophotometric titration experiment using Benesi-Hildebrand plot (Fig. 7d). Again, upon addition of Cu^{2+} to the solution mixture having S^{2-}, restores the pink color of the solution and the absorption peak at 523 nm reappears. The IR spectra of P + Cu^{2+}+S^{2-} is in agreement with the spectra obtained for P + Cu^{2+} confirming the recyclability as shown in Fig. S13. Partial attachment of the S^{2-} ion to the Cu^{2+} ion leads to the formation of CuS and thus regenerates the cyclic spirform of the probe [41]. This reveals that signal process is reversible; hence, the chemosensor would be recyclable (Fig. S12).

3.8. Theoretical calculations

The probable binding fashion of the probe to Cu^{2+} was also explored with density functional theory (DFT) calculations. B3LYP method and general basis sets were used to optimize all geometries. Optimized structure of **P** and **P** + Cu^{2+} complex has shown in Fig. 7a and c respectively. HOMO of the probe suggests that a high electronic density resides on O, N atoms and thus these are the favorable binding site for the metal ions. As predicted, Cu^{2+} ion prefers to bind with O and N atoms and alpha and beta HOMO of **P** + Cu^{2+} complex is shown in Fig. 7d, e. Study revealed that the **P** + Cu^{2+} complex is −35.64 eV more stable than separated reactants.

3.9. Mechanism of sensing

To understand the sensing behavior, we decided to analyze it by FTIR, LCMS, XPS and density functional theory (DFT) calculation. FTIR was used to examine the influence of C═O bond on binding Cu^{2+} ions. The peak at 1690 cm^{-1} is the characteristic stretching frequency for the C═O bond of the rhodamine unit. Upon binding to Cu^{2+} the peak at 1690 cm^{-1} disappears; suggest the breaking of C═O and the Cu^{2+} bonding with O-atom (Fig. S9). XPS characterization of probe and **P** + Cu^{2+} complex had been carried out to understand the fate of Cu^{2+} ion with probe (Fig. S10). Cu2p spectrum of the probe suggests the absence of copper ions (Fig. S10d). Peaks at 933.5 and 953.2 eV in Cu2p spectrum of **P** + Cu^{2+} complex corresponds to $Cu2p_{3/2}$ and $Cu2p_{1/2}$ respectively (Fig. S10). The peaks at 940.2, 944.5 eV corresponds to the strong satellite peak which confirms that Cu is in (II) oxidation state. The peak at 961.9 eV suggests the binding of Cu^{2+} with O-atom [42]. The N1 s peak becomes symmetrical in the **P** + Cu^{2+}complex (Fig. S10f) and shifts towards higher binding energy by 0.7 eV as compared to the bare probe (Fig. S10b) due to its interaction with Cu^{2+} ion. Shifting of N1s and O1s peak of **P** + Cu^{2+} complex as compared to probe towards higher binding energy suggests coordination of these atoms with Cu^{2+} ions. Additionally, to explore the colorimetric "On-Off" characteristics, the LCMS spectrum of the system [**P** + Cu^{2+} +S^{2-}] was analyzed. In brief, after addition of S^{2-} solution to the **P** + Cu^{2+}, the resultant solution was centrifuged at 12,000 rpm for 30 min to remove the formed CuS. The supernatant was analyzed for LCMS. The peak at 563.27 confirm the formation of free probe upon addition of S^{2-} (mass of probe: 563.25 and mass of **P** + Cu^{2+}: 624.06) (Fig. S11). It is evident from Cu^{2+} binding studies that the binding leads to opening of the spirolactam ring of probe and that cause the color change. As demonstrated in Scheme 2, the addition of Cu^{2+} ion initiates colorimetric "On" response, generating a deep pink color.

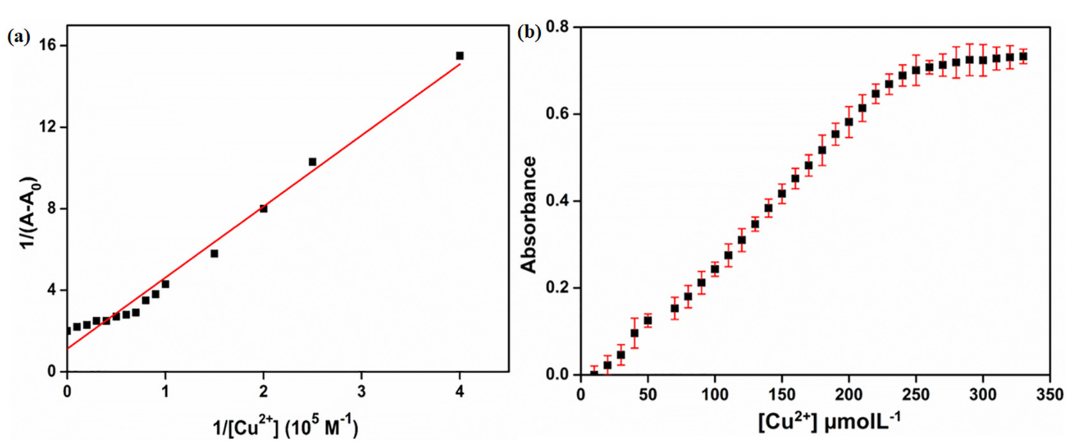


Fig. 5. (a) Benesi-Hildebrand plot. (b) Limit of detection calibration graph.

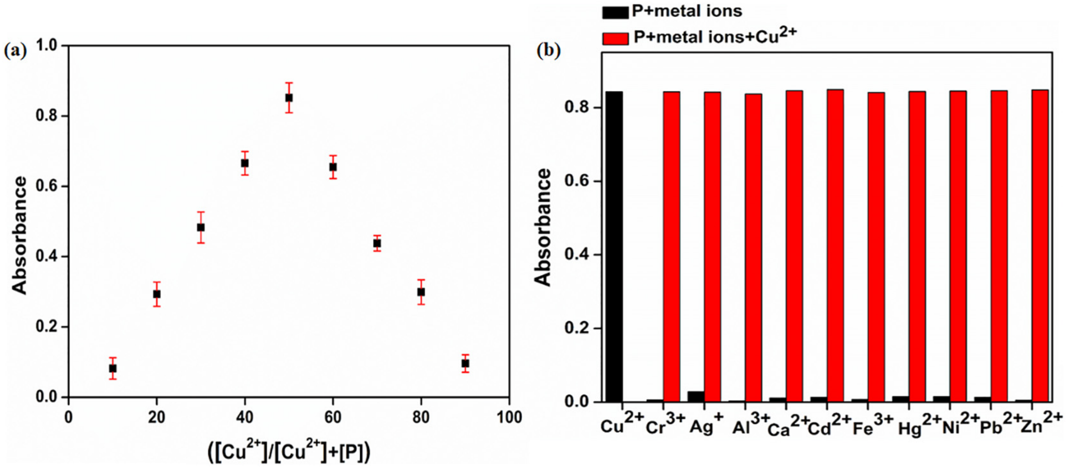


Fig. 6. (a) Job's plot (b) Effect of interfering cations to the absorption of the P + Cu^{2+} complex.

Also, the added S^{2-} can apprehend Cu^{2+} ion, and induce a colorimetric change from deep pink to colorless (Fig. 8a, inset).

4. Applications of probe

To assess the performance of a designed probe, real sample analysis is remarkable because of its viable impact from naturally occurring substances. The real-time implication of the probe was appraised through the determination of Cu^{2+} ions in tap water, drinking water, industrial water and human blood samples. The water and blood samples were also analyzed for Cu^{2+} and validated by AAS and ICP-OES methods respectively. Samples were examined with their three replicates. The results acquired from this chemosensor are summarized in Tables 1 and 2. It can be seen from Tables 1 and 2, that the results obtained for water and human blood samples are in well accordance with AAS and ICP-OES. Therefore, the proposed colorimetric chemosensor has good practical feasibility in quantitative detection of copper in different environmental and biological samples. Therefore, the proposed colorimetric chemosensor has good practical feasibility in determination of copper in divergent samples.

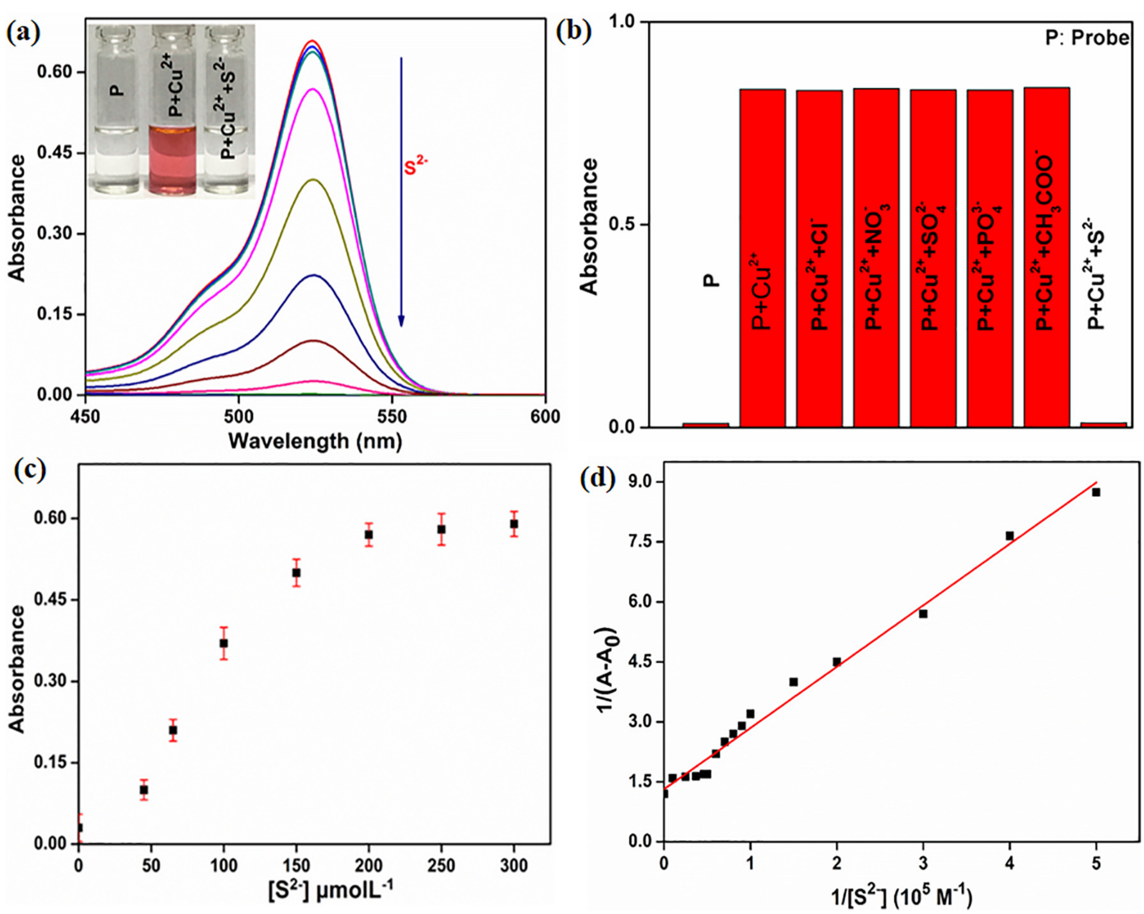
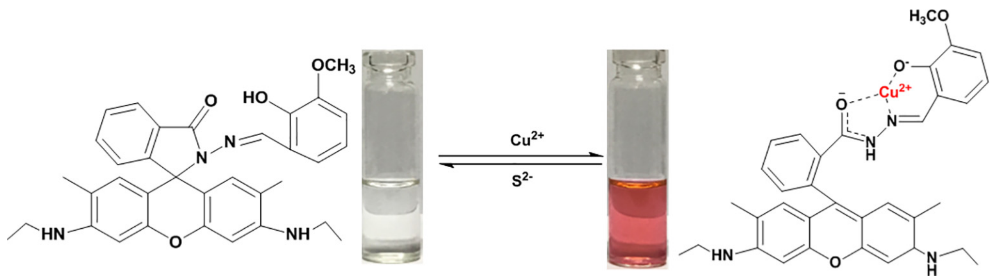


Fig. 7. (a) UV–Vis spectra showing reversibility of P + Cu^{2+} complex upon addition of S^{2-}. (b) Effect of anions to the absorption of the P + Cu^{2+} complex. (c) Limit of detection calibration graph. (d) Benesi-Hildebrand plot.



Scheme 2. Proposed mechanism for the absorption changes in probe upon addition of Cu^{2+} and S^{2-}.

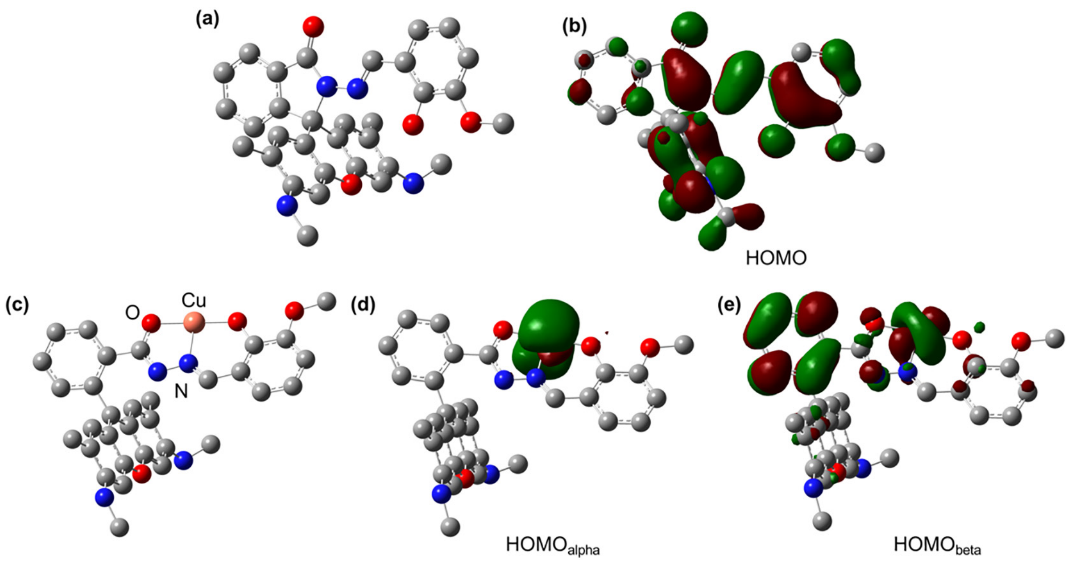


Fig. 8. (a) Optimized structure and (b) HOMO of P; (c) Optimized structure (d, e) alpha and beta HOMO of P + Cu^{2+} complex respectively.

Table 1
Determination of Cu^{2+} in water samples.

Samples	AAS method		Present method	
	Cu^{2+} found	RSD*(%)	Cu^{2+} found	SE
Tap water	7.74×10^{-7} M	1.27	7.91×10^{-7} M	1.52
Drinking water	7.56×10^{-7} M	2.84	7.21×10^{-7} M	0.68
Industrial water	1.50×10^{-7} M	2.07	1.31×10^{-6} M	1.47

5. Test strips for quick detection of Cu^{2+}

Requirements for practical applications, portable testing strips were fabricated. The test strips were prepared by coating the nitrocellulose paper with PVA. The PVA coating neutralizes the charge on the surface of membrane partially and blocks the probe through hydrogen bonding hence making it viable for coating of the probe and then dried in air [43].

Table 2
Determination of Cu^{2+} in blood samples.

Samples	ICP-OES method		Present method	
	Cu^{2+} found	RSD*(%)	Cu^{2+} found	SE
CBRM 1	5.27×10^{-7} M	5.24	5.98×10^{-7} M	1.94
CBRM 2	5.27×10^{-7} M	2.70	5.86×10^{-7} M	1.17
BS 1	1.29×10^{-6} M	0.03	1.20×10^{-6} M	0.88
BS 2	1.40×10^{-6} M	0.66	1.31×10^{-6} M	0.72

These test strips were directly used in the detection of Cu^{2+} solutions in various concentrations. The color change of the strips was observed only after treating them with Cu^{2+} solution (Fig. 9). The color change suggests the probe could be employed as portable tool for rapid and naked eye detection of Cu^{2+}.

Table 3 documented lately published research works on Cu^{2+} chemosensors. Our synthesized chemosensor has fairly high LOD, is highly sensitive and selective to Cu^{2+} ion.

6. Conclusion

We have reported an o-Vanillin functionalized Rhodamine 6G Hydrazide derivative as probe which exhibits high selectivity and sensitivity towards Cu^{2+} and over other important ions studied, viz., Hg^{2+}, Cd^{2}

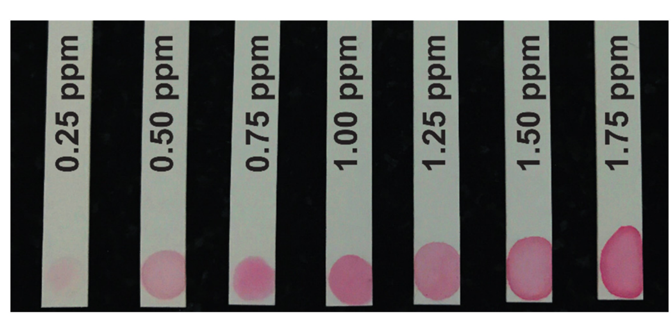


Fig. 9. Test strips for quick detection of Cu^{2+} (from L to R: concentration of Cu^{2+}).

Table 3
Comparative literature reports for Cu^{2+} ion detection.

Sensing method	LOD for Cu^{2+}	Reference
Colorimetric	2.0×10^{-6} M	[44]
Fluorometric	3.9×10^{-6} M	[45]
Colorimetric	2.9×10^{-6} M	[21b]
Fluorometric	2.3×10^{-6} M	[46]
Colorimetric	5.0×10^{-6} M	[47]
Colorimetric	2.9×10^{-6} M	[48]
Colorimetric	7.3×10^{-10} M	[22b]
Fluorometric	3.9×10^{-6} M	[45]
Colorimetric	1.2×10^{-6} M	[49]
Colorimetric	7.2×10^{-7} M	[11e]
Colorimetric	1.8×10^{-8} M	Present work

$^{+}$, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, Ag^{+}, Cr^{3+}, Ca^{2+}, Al^{3+}, Fe^{3+}, and Pb^{2+}, as manifested by titrations of individual as well as interfering metal ions. Interaction of the Cu^{2+} with probe enhances the absorption intensity at 523 nm and causes a "turn-On" colorimetric response in the visible region and shows limit of detection to be 1.8×10^{-8} M. The sensing mechanism of the probe has been evidenced by UV–Vis absorption, FTIR and LCMS spectroscopy. The binding mechanism elucidated from DFT studies revealed that the **P** + Cu^{2+} complex is −35.64 eV more stable than individual reactants. The test strips made from probe could be employed as portable tool for rapid and naked eye detection of Cu^{2+}. The reversible nature of the probe with high sensitivity may lead to the potential applications for the qualitative and quantitative detection of trace amounts of Cu^{2+} in various chemical, environmental and biological samples.

Declaration of Competing Interest

There are no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2019.117291.

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