Dipicolinimidamide functionalized chromogenic chemosensor for recognition of Cu²⁺ ions and its applications

Prashant A. Patil [a],[*], Suman Sehlangia [b], Chullikkattil P. Pradeep [b]

[a] S.S.V.P.S's L. K. Dr. P. R. Ghogrey Science College, Dhule, 424 005, M.S, India
[b] School of Basic Sciences, Indian Institute of Technology, Mandi, 175001, Himachal Pradesh, India

ARTICLE INFO

ABSTRACT

A new dipicolinimidamide based receptor, N,N'-(pyridine-2,6-diyl)dipicolinimidamide receptor **AM2** is designed for the recognition of Cu²⁺ among all cations with high selectivity investigated in aqueous solutions *via* synergistic effects of intermolecular hydrogen bonding and electrostatic interactions. The photophysical properties of the **AM2** receptor were tested by UV/Visible absorption methods. The binding method was completely confirmed by computer based studies. Theoretical statistics have highlighted the role of π-π stacking and hydrogen bonding in binding of Cu²⁺ with receptor **AM2**. Receptor **AM2** selectively recognized Cu²⁺ in EtOH/water with a detection limit down to 3.49 μM and can be utilized to recognize Cu²⁺ ions in real samples.

1. Introduction

Molecular chemosensors are able to connect specific ionic and biomolecular types by selecting that the attachment state comes out with a computable signal, especially changes in the appearance of chromophores or fluorophores. Development of chemosensor for the competent recognition of cations and anions are an active area of research in the field of chemical, biology and environmental sciences [1–9]. In the recent year's, remarkable interest has been focused on the formation and synthesis of chemosensors to detect d-block metal ions because of the pronounced importance of these ions in medical, biological and environmental applications [10–13]. Amongst the transition metal ions, Cu²⁺ is the third most important component of the human body and plays a key job in a variety of physiological processes like biosynthesis of hemoglobin, bone growth, dopamine production, regulation of neurological function, genetic expression and the functional and structural enrichment of proteins [14–17].Conversely, "An excess of Cu²⁺ ion is quite unsafe to living systems and it's overaccumulation in human body leads to various neurodegenerative diseases such as amyotrophic sclerosis, Alzheimer's disease, prion disease, Wilson's disease, Menke's disease, kidney damage, lipid metabolism and gastrointestinal disorders,

inflammatory disorders" [18–20]. Several techniques were reported for the recognition of Cu²⁺ ions counting AAS (atomic absorption spectrometry), ICP-AES (Inductively coupled plasma atomic emission spectrometry, ICP-MS (Inductively coupled plasma mass spectroscopy), cyclic voltammetry etc., but these techniques have major limitation of requirement of highly sophisticated pricey instrumentation, tedious sample preparation and skilled operator [21–24]. Subsequently, Cu²⁺ is a fluorescence quencher due to its paramagnetic nature and therefore most of the fluorescence chemosensors for Cu²⁺ shows turn-off response rather than turn-on response, rendering stumpy indication. Hence, fluorescent sensors may be not as much suitable for molecular detection mainly for Cu²⁺, sometimes they give a false positive response which limits their application [25–28]. Alternatively, colorimetric chemosensors are well-suited for the analyte and are generally used due to the low down cost and lack of costly utensils required, as well as color changes that can be observed directly with the naked eye, even if tested extensively [29, 30].

The previous management of these things in mind and as a part of our uninterrupted research [33–37], we designed a 2, 6-diaminopyridine conjugated with 2-Cyanopyridine in presence of SnCl₄ through dipicolinimidamide linkage. The two nitrogens and NH provides hydrogen

bonding combinations that can be used for binding selectively with the appropriate analyte. Here, we report the synthesis and spectral evaluation of N, N '- (pyridine-2, 6-diyl) dipicolinimidamide (**AM2**) and improve Cu^{2+} sensitivity. Additional sensitivity of Cu^{2+} was tested using a visible UV titration test at an aqueous solvent. The discriminating and receptive nature of the **AM2** receptor toward Cu^{2+} arises from electrostatic interactions between nitrogen containing functional groups. The receptor **AM2** could be powerful candidate as a practical colorimetric sensor for Cu^{2+} ions.

2. Materials and methods

2.1. Experimental

All reactions were performed using glassware dried in the oven under good nitrogen pressure unless otherwise specified. Where necessary, solvents were distilled before use. Sigma Aldrich, India brand was selected to buy all chemicals. The PerkinElmer Spectrum One FT-IR spectrometer was used to record IR spectra as KBr pellets and nujol mulls. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were determined on a Bruker AVANCE II 400 spectrometer. Agilent's LCMS instruments were used to carry out LC-MS analysis. Additionally, U-3900 spectrophotometer (PerkinElmer Co., USA) was used to perform all UV Visible spectral experiments and perchlorate salt of metals used in study.

2.2. Spectroscopic studies

Studies of detecting cations by receptor **AM2** were performed at moderate temperatures. All solutions were thoroughly stirred to ensure consistency before the absorption spectral experimentation. The **AM2** receptor was soluble in ethanol: water solvent system and therefore **AM2** stock solutions (1.0×10^{-3} M) were prepared with ethanol: water (10:90 *v/v*). All solutions for cations (1.0×10^{-2} M) were prepared in doubly purified water. After appropriate dilution, these solutions were applied to all spectroscopic investigations. The cuvette was filled directly with the requisite volume of diluted **AM2** receptor (2 ml, 4.0×10^{-5} M, in H_2O) and the spectra was recorded after each subsequent injection of Cu^{2+} ions (0–400 µL, 1×10^{-3} M, in H_2O) using a micropipette for spectroscopic (UV–Vis) activities.

2.3. Synthesis of receptor AM2

The synthesis of receptor **AM2** was carried out based on previously reported methods [31,32,38]. In 100 ml dry round bottom flask, a mixture of 2, 6-diaminopyridine (1.09 gm, 10 mmol) and 2-Cyanopyridine (1.92 gm, 20 mmol) in THF (15 mL) solvent was under magnetic stirring. The resulting mixture was stirred at 140 °C for one and half hours under oil bath with constant stirring. After 30 min, $SnCl_4$ (2.2 mL, 24 mmol) was added to the reaction mixture. After the addition of $SnCl_4$, temperature was enhanced to 150–160 °C, and heated up for 3–4 Hours. The movement of reaction was supervised by of TLC. After the reaction is complete, the product is cooled to room temperature and then dissolved in warmed water with an alkaline 10% sodium hydroxide solution. Dichloromethane [3 × 100] was employed to extract the alkaline solution. The activated charcoal was used to decolorize the organic layer and which subsequently dried over anhydrous sodium sulphate. The amidine was obtained a crude product after evaporating the solvent under reduced pressure. Recrystallization of the crude product was achieved from acetone and hexane mixture system that afforded crystalline product. Yield: 68%; Mol. Formula: $C_{17}H_{15}N_7$; Mol. Weight: 317 g; Physical Nature: Yellow needles; FT-IR spectrum (cm^{-1}) [KBr]: 3369.7, 3182.6, 1400.3–1450.5; Mass (ESI +ve): 318 (M+1), ^1H NMR (400 MHz, δ ppm):6.92–6.95 (m, 2H), 7.26–7.29 (m, 2H), 7.37–7.40 (m, 2H), 7.64–7.67(m, 2H), 7.80–7.87 (m, 2H), 8.38(d, 1H),8.54(dd, 2H),8.60(d, 1H),10.32 (s, 1H).^{13}C NMR (500 MHz,δ ppm):167.7, 161.1, 157.8, 149.6, 139.5, 137.1, 126.6, 97.6.

2.4. Computational study

The formal design of **AM2** and its Cu^{2+} guest facilities is done using the Gaussian 09W [41] computer system using a density functional theory (DFT) method. All DFT computation were carried out in the gas phase with active B3LYP (Becke's parameter hybrid functional using LYP correlation functional) using a 6-31G (d, p) base for C, H, N, O atom and LANL2DZ Cu atom.

3. Results and discussion

The receptor N, N'-(pyridine-2, 6-diyl)dipicolinimidamide(**AM2**) was prepared by treating 2-Cyanopyridine and 2,6-Diaminopyridine using of $SnCl_4$ as a lewis acid to afford **AM2**(Scheme 1).The structure of receptor **AM2** was confirmed with the help of ^1H NMR, ^{13}C NMR and LC-MS spectroscopic techniques (Fig. S1-3, SI). The naked-eye noticeable color change of **AM2** (2 mL, 1.0×10^{-3} M in 10:90 v/v EtOH:H_2O) was experienced by adding an equivalent quantity of diverse cations like Ca^{2+}, Ba^{2+}, Cd^{2+}, Sr^{2+}, Cs^+, Cr^{3+}, Na^+, Ni^{2+}, Li^+, Zn^{2+}, Co^{2+}, K^+, Mn^{2+}, Al^{3+}, Ag^+, Pb^{2+}, Mg^{2+}, Hg^{2+}, Fe^{2+} and Fe^{3+}(1ml, 1.0×10^{-2} M, in H_2O)(Fig. 1). In the presence of Cu^{2+} only, the receptor **AM2** showed a detectable color transform from colorless to yellow.

The UV–visible spectroscopic performances of receptor **AM2** (1.0×10^{-5} M) were first examined upon addition of various cations (1.0×10^{-2} M) like Co^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Zr^{2+}, Cs^+, Cd^{2+}, Ca^{2+}, Ba^+, Fe^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}, K^+, Al^{3+}, Sr^{2+} and Na^+ in water. The receptor **AM2** (1.0×10^{-5} M) exhibits a strong peak at 279 nm. At 279 nm, the absorption tip of pure receptor **AM2** was red shifted to 290 nm due to intramolecular charge transfer (ICT) effect upon addition of 1 equivalent Cu^{2+} (Fig. 2). A red shift in UV–Vis absorption of the **AM2** receptor was also noted in the titration study (Fig. 3), in which Cu^{2+} ions (0–10 equivalents) were added sequentially to the fixed concentration of the **AM2** receptor (1.0×10^{-5} M). The spectral alterations have clearly explained that complex formation have occurred between the **AM2** and Cu^{2+} receptors. No visible color changes and spectral changes of **AM2** were detected in the presence of other tested cations. The charge transfer stimulates the color as well as spectral changes of **AM2** receptor, apparently due to the delocalization of electrons from pyridine-N, amidine-N to Cu^{2+} during the formation of complex.

From the UV–visible titrations, the association constant of 2421.7 M^{-1} was calculated by Bensi-Hildbrand method for Cu^{2+} (Fig. S4) [40]. In the EtOH/H_2O (10:90, *v/v*) solvent system, the calculated limit of detection for Cu^{2+} was 3.49 µM determined with the help of standard IUPAC method of 3σ method (Fig. S5), which is lower than WHO allowed limit.

The stoichiometry of **AM2** with Cu^{2+} could be estimated to be 2:1 which determined by Job's Method of Continuous Variation [39].The plot of mole ratio (Fig. S6) and the Job's plot (Fig. S7) support the development of a complex between **AM2** and Cu^{2+} with 2: 1 binding stoichiometry. Additionally, the direct confirmation for the formation of a 2: 1 complex was achieved from the LC-MS spectra of **AM2** in existence of 2.0 equivalents Cu^{2+} in water. This data was additionally confirmed by mass spectral examination. The mass data validates the formation 2:1 complex between double deprotonated **AM2** and metal ion. The main characteristic MS peak was observed at *m/z (for $C_{17}H_{15}N_7$)* = 318.4 (M+1) for pure **AM2**.(Fig. S3).Conversely, upon addition of 2.0 equivalents of Cu^{2+}, the peak at 318.4 vanished and a novel peak come into view at *m/z* = 746.198 matching to the complex $[(M-2H) + 2Cu^{2+} + 2(ClO_4^-) + 6 H_2O + H]^+$(Fig. S8). The receptor **AM2** can bind to the Cu^{2+} ion via binding sites consisting of nitrogen atoms of terminal pyridine nucleus and the imino group of two amidine linkage. The lone pair of electrons on the nitrogen atoms of the terminal pyridine ring and imino groups of the receptor **AM2** can be delocalized from an orbital containing an electron pair to the vacant orbital localized on the Cu^{2+}. This coordination is responsible for the color change from colorless to clear yellow. Further, ^1H NMR titration experiment was carried out to confirm the
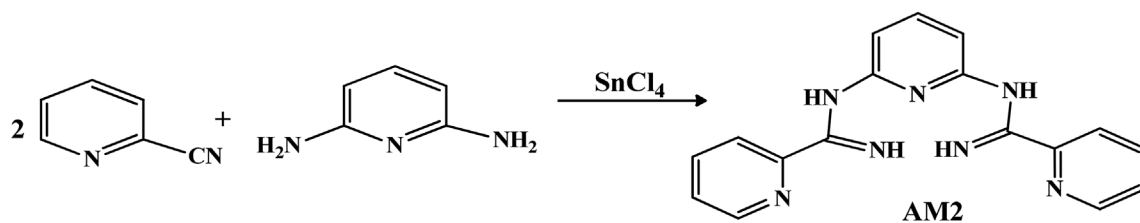
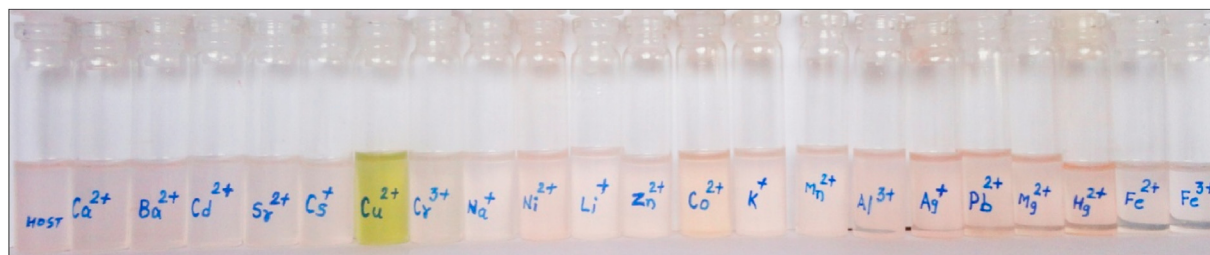**Scheme 1. Synthesis of Receptor AM2.**

Fig. 1. The color changes of **AM2** (1.0×10^{-5} M,10:90 *v/v* EtOH:H$_2$O) upon addition of equivalent amount of different cations in H$_2$O. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
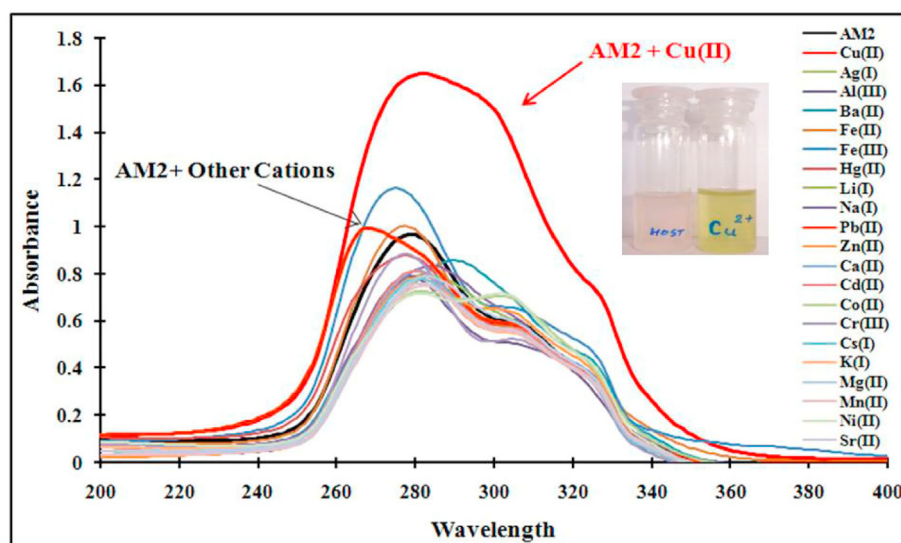

Fig. 2. UV–Vis spectra changes of the receptor **AM2** (1×10^{-5} M) upon addition of various cations (1×10^{-2} M) in EtOH: H$_2$O.

nature of the interaction of the receptor **AM2** in CDCl$_3$ solution with different concentration of copper (II) perchlorate in MeOH-d$_4$ solvent (Fig. S9). From the titration experiments, it is observed that peak at 10.316 ppm corresponding to the –NH proton of amidine-imine moiety gets disappeared on successive addition of Cu^{2+} ion in the solution of **AM2**. This indicates a shift of a nitrogen lone pair towards the metal ions.

The possible 3D structure (Fig. 4) and the coordination occurring all through the encapsulation of Cu^{2+} by **AM2** were scrutinized by density functional theory (DFT) calculations. The optimized structure of **AM2** and its complex with Cu^{2+} are shown in Fig. 4 (a). It is observed that the energy gap of both HOMO and LUMO reduces in **AM2-Cu** system, probably due to the higher degree of stabilization than the former **AM2** system and resulting an overall decrease of the gap from 0.15886 eV to 0.08142 eV.The figure also exposes that the charge density at HOMO of **AM2** is spread over throughout the molecule and upon complexation, with copper ion, the charge density is shifted towards terminal pyridine moiety.

Further, to get analytical application of sensing possessions of receptor **AM2** toward Cu^{2+}, the absorbance of different competitive ions in EtOH/H$_2$O (10:90, *v/v*) solution was scrutinized, and the consequences were shown in Fig. 5. No considerable changes were observed when Cd^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Zr^{2+}, Cs$^+$, Cd^{2+}, Ca^{2+}, Ba$^+$, Fe^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}, K$^+$, Al^{3+}, Sr^{2+}, Na$^+$ were added in the receptor **AM2**solution even at high concentration (10 equiv). Competition experimentations in the addition of Cu^{2+} (1 equiv) showed enhancement. This result evidently pointed out the specificity of the receptor **AM2** to detect Cu^{2+} under competitive environment.

4. Practical applications of AM2

To ensure that **AM2** is practically applicable, the **AM2** loaded test strips were designed to sense Cu^{2+} ions from an aqueous media. The preferred test strips were organized by absorbing small pieces of cellulose paper (Whatman No. 42) in an **AM2** solution (1.0×10^{-3} M) in methanol
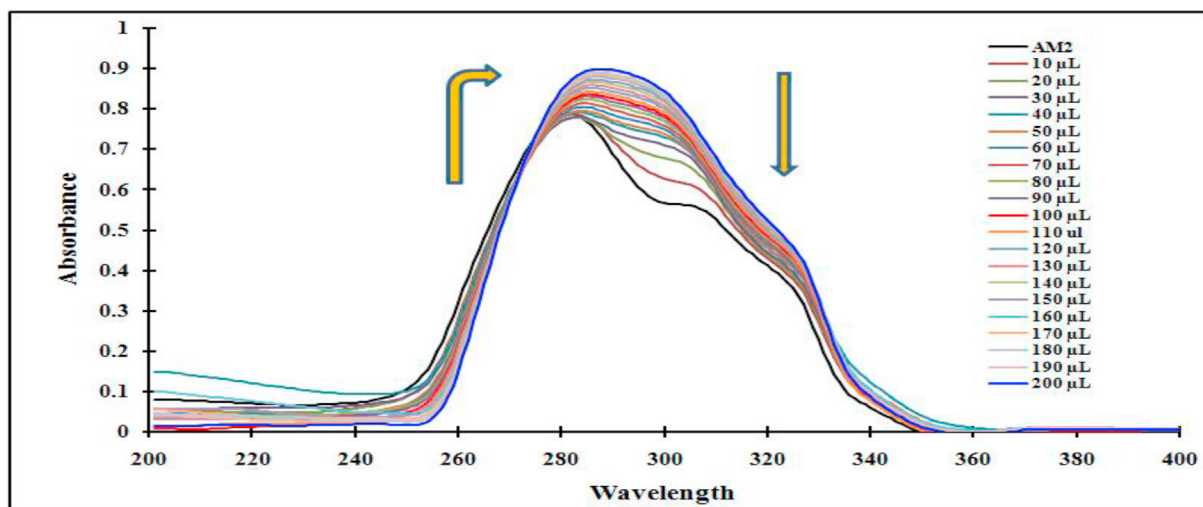
Fig. 3. Absorption spectra of **AM2** (1.0×10^{-5} M) upon incremental addition of Cu^{2+} ions (1–10 equiv, 1.0×10^{-2} M, in H_2O).
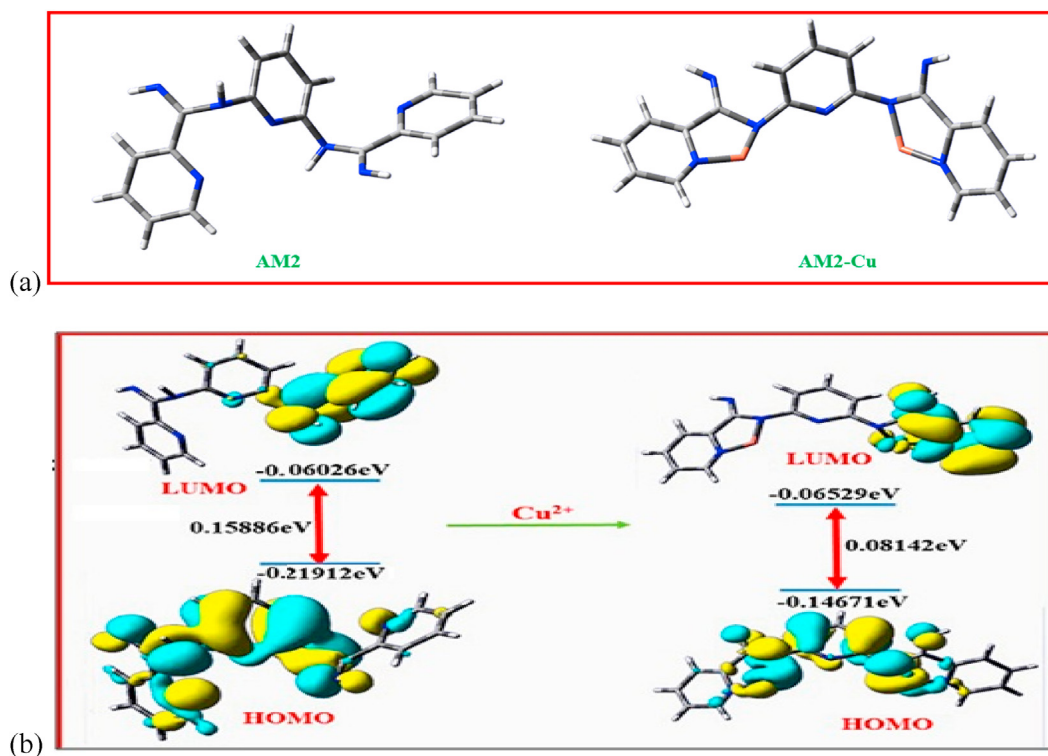

Fig. 4. DFT computed (a) optimized structure of **AM2** and **AM2**.Cu^{2+} complex (b) LUMO-HOMO diagrams of **AM2** and **AM2**.Cu^{2+} complex.

and were dried in air. When these strips were treated with a 10 mL solution containing Cu^{2+} (1.0×10^{-2} M), the monotonous strip turned yellow (Fig. 6 A). The quick color transformation of the test strip in solution defined the effective use of **AM2**. The detection of Cu^{2+} by **AM2** also operated with solid support. Silica gel (60–120 mesh, 5.0 g, colorless) was dipped in **AM2** (methanol, 5 mL, 1.0×10^{-3} M) and the solvent removed. A dark green color of silica gel appeared, indicating the adsorption of the receptor on the surface. When treated with a 10 mL solution containing Cu^{2+} (1.0×10^{-2} M), the colorless solution quickly turned to a orange-yellow color (Fig. 6B). The rapid color change of the solid silica gel in the solution highlighted the effective use of **AM2** for qualitative recognition of Cu^{2+} in aqueous environment.

5. Conclusion

In summing up, we demonstrated the synthesis and use of **AM2** as a new chromogenic chemosensor for the selective recognition of Cu^{2+} ions in pure aqueous environment. The sensor display high selectivity and high sensitivity towards Cu^{2+} in aqueous environment. The detection of Cu^{2+} induces a spectacular color distinction of **AM2** from colorless to yellow that permitting naked eye recognition. Besides, the recognition of Cu^{2+} in aqueous media by **AM2** can be revealed by paper strip and a solid supported method using silica. These methods promote seamless and fast detection of Cu^{2+} in aqueous environment with a detection limit of up to 10 µM. The excellent selectivity of sensors **AM2** towards Cu^{2+} in a pure
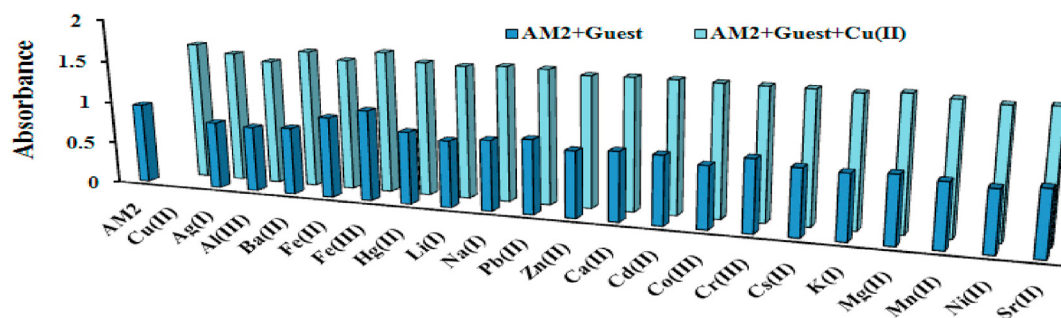
Fig. 5. Competitive responses of **AM2** and **AM2**:Cu^{2+} complex at 282 nm in absorbance intensity.
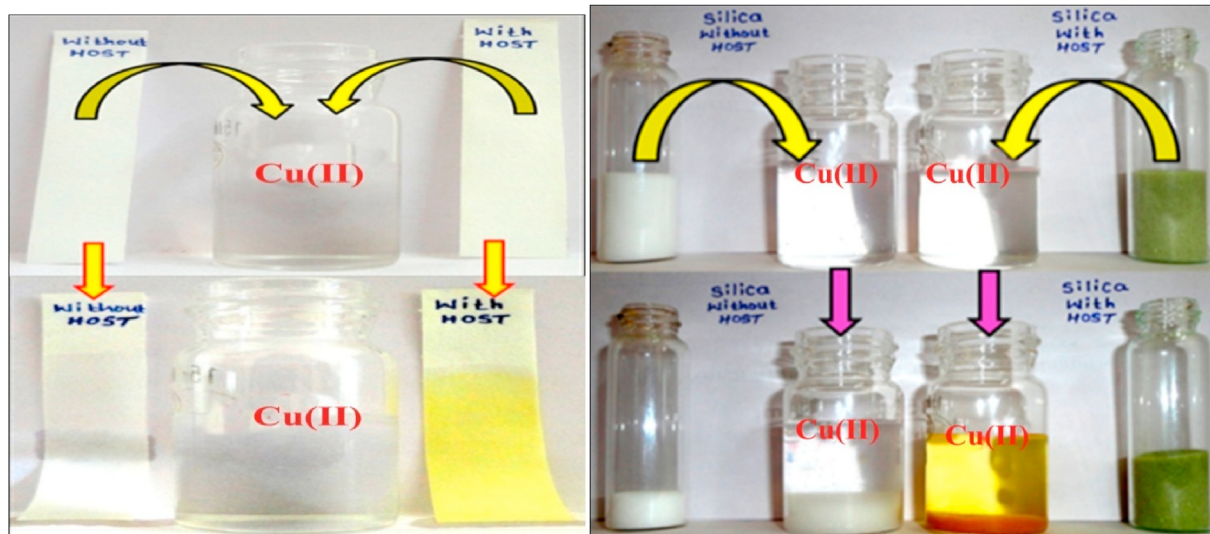

Fig. 6. Practical application of **AM2** for the detection of Cu^{2+} by: (A) test strip method and (B) silica support method.

aqueous environment makes **AM2** a promising candidate for qualitative as well as quantitative recognition of Cu^{2+} in a variety of chemical and environmental applications.

Declaration of competing interest

I hereby declared that authors in the above paper have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sintl.2020.100075.

References

[1] M. Beija, C.A.M. Afonso, J.M.G. Martinho, Synthesis and applications of Rhodamine derivatives as fluorescent probes, Chem. Soc. Rev. 38 (2009) 2410–2433.

[2] D.G. Cho, J.L. Sessler, Modern reaction-based indicator systems, Chem. Soc. Rev. 38 (2009) 1647–1662.

[3] W.S. Han, H.Y. Lee, S.H. Jung, S.J. Lee, J.H. Jung, Silica-based chromogenic and fluorogenic hybrid chemosensor materials, Chem. Soc. Rev. 38 (2009) 1904–1915.

[4] N. Johnsson, K. Johnsson, Chemical tools for biomolecular imaging, ACS Chem. Biol. 2 (2007) 31–38.

[5] A.A. Martí, S. Jockusch, N. Stevens, J.Y. Ju, N.J. Turro, Fluorescent hybridization probes for sensitive and selective DNA and RNA detection, Acc. Chem. Res. 40 (2007) 402–409.

[6] E.M. Nolan, S.J. Lippard, Small-molecule fluorescent sensors for investigating zinc metalloneurochemistry, Acc. Chem. Res. 42 (2009) 193–203.

[7] S.W. Thomas, G.D. Joly, T.M. Swager, Chemical sensors based on amplifying fluorescent conjugated polymers, Chem. Rev. 107 (2007) 1339–1386.

[8] O.S. Wolfbeis, Materials for fluorescence-based optical chemical sensors, J. Mater. Chem. 15 (2005) 2657–2669.

[9] a) Z. Xu, X. Chen, H.N. Kim, J. Yoon, Sensors for the optical detection of cyanide ion, Chem. Soc. Rev. 39 (2010) 127–137;
b) X.T. Zheng, Y.N. Tan, Recent development of nucleic acid nanosensors to detect sequence-specific binding interactions: from metal ions, small molecules to proteins and pathogens, Sensor Int. 1 (2020) 100034.

[10] C. Bargossi, M.C. Fiorini, M. Montalti, L. Prodi, N. Zaccheroni, Recent developments in transition metal ion detection by luminescent chemosensors, Coord. Chem. Rev. 208 (2000) 17–32.

[11] E.M. Nolan, S.J. Lippard, Tools and tactics for the optical detection of mercuric ion, Chem. Rev. (108) (2008) 3443–3480.

[12] L. Prodi, F. Bolletta, M. Mont*zcxvalti, N. Zaccheroni, Luminescent chemosensors for transition metal ions, Coord. Chem. Rev. 205 (2000) 59–83.

[13] B. Valeur, I. Leray, Design principles of fluorescent molecular sensors for cation recognition, Coord. Chem. Rev. 205 (2000) 3–40.

[14] C. Andreini, L. Banci, I. Bertini, A. Rosato, Occurrence of copper proteins through the three domains of life: a bioinformatic approach, J. Proteome Res. 7 (2008) 209–216.

[15] E.D. Harris, Copper and Iron: a landmark connection of two essential metals, J. Trace Elem. Exp. Med. 14 (2001) 207–210.

[16] L. Huang, X. Wang, G. Xie, P. Xi, Z. Li, M. Xu, Y. Wu, D. Bai, Z. Zeng, A new rhodamine-based chemosensor for Cu^{2+} and the study of its behaviour in living cells, Dalton Trans. 39 (2010) 7894–7896.

[17] E. L Que, D.W. Domaille, C. Chang, Metals in neurobiology: probing their chemistry and biology with molecular imaging, Chem. Rev. 108 (2008) 1517–1549.

[18] E. Gaggelli, H. Kozlowski, D. Valensin, G. Valensin, Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis), Chem. Rev. 106 (2006) 1995–2044.

[19] G. Muthaup, A. Schlicksupp, L. Hess, D. Beher, T. Ruppert, C.L. Masters, K. Beyreuther, The amyloid precursor protein of Alzheimer's disease in the reduction of copper (II) to copper (I), Science 271 (1996) 1406–1409.

[20] D. Strausak, J.F.B. Mercer, H.H. Dieter, W. Stremmel, G. Multhaup, Copper in disorders with neurological symptoms: alzheimer's, Menkes, and Wilson diseases, Brain Res. Bull. 55 (2001) 175–185.

[21] J.S. Becker, M. V Zoriy, C. Pickhardt, N. Palomero-Gallagher, K. Zilles, Imaging of copper, zinc and other elements in thin section of human brain samples, Anal. Chem. 77 (2005) 3208–3216.

[22] Y. Liu, P. Liang, L. Guo, Nanometer titanium dioxide immobilized on silica gel as sorbent for preconcentration of metal ions prior to their determination by inductively coupled plasma atomic emission spectrometry, Talanta 68 (2005) 25–30.

[23] J. Otero-Romani, A. Moreda-Pineiro, A. Bermejo-Barrera, P. Bermejo-Barrera, Evaluation of commercial C18 cartridges for trace elements solid phase extraction from seawater followed by inductively coupled plasma-optical emission spectrometry determination, Anal. Chim. Acta 536 (2005) 213–216.

[24] N. Pourreza, R. Hoveizavi, Simultaneous preconcentration of Cu, Fe and Pb as methylthymol blue complexes on naphthalene adsorbent and flame atomic absorption determination, Anal. Chim. Acta 549 (2005) 124–128.

[25] J. Hou, L.-Y. Wang, D.-H. Li, X. Wu, A rigid conjugated pyridinylthiazole derivative and its nanoparticles for divalent copper fluorescent sensing in aqueous media, Tetrahedron Lett. 52 (2011) 2710–2714.

[26] R. Tang, K. Lei, K. Chen, H. Zhao, J. Chen, A rhodamine-based off on fluorescent chemosensor for selectively sensing Cu (II) in aqueous solution, J. Fluoresc. 21 (2011) 141–148.

[27] L. Yang, Q. Song, K. Damit-Og, H. Cao, Synthesis and spectral investigation of a Turn-On fluorescence sensor with high affinity to Cu2+, Sens. Actuators, B 176 (2013) 181–185.

[28] C. Yu, L. Chen, J. Zhang, J. Li, P. Liu, W. Wang, B. Yan, "Off-On" based fluorescent chemosensor for Cu2+ in aqueous media and living cells, Talanta 85 (2011) 1627–1633.

[29] C. Guo, J. Wang, J. Cheng, Z. Dai, Determination of trace copper ions with ultrahigh sensitivity and selectivity utilizing Cd Te quantum dots coupled with enzyme inhibition, Biosens. Bioelectron. 36 (2012) 69–74.

[30] Y. Wang, L. Wang, L.L. Shi, Z.B. Shang, Z. Zhang, W.J. Jin, Colorimetric and fluorescence sensing of Cu2+ in water using 1, 8-dihydroxyanthraquinone-β-cyclodextrin complex with the assistance of ammonia, Talanta 94 (2012) 172–177.

[31] F.H.G. da-Silva, D.G.J. Batista, M.B. Meuser, K.C. Demarque, T.O. Fulco, J.S. Araújo, P.B. Da Silva, C.F. Da Silva, D.A. Patrick, S.M. Bakunova, S.A. Bakunov, R.R. Tidwell, G.M. Oliveira, C. Britto, O.C. Moreira, M.N.C. Soeiro, In vitro and in vivo trypanosomicidal action of novel arylimidamides against trypanosoma cruzi, Antimicrob. Agents Chemother. 60 (2016) 2425–2434.

[32] S.M. Sondhi, R. Rani, P. Roy, S.K. Agrawal, A.K. Saxena, Synthesis, anti-inflammatory, and anticancer activity evaluation of some heterocyclic amidine and bis-amidine derivatives, J. Heterocycl. Chem. 48 (2011) 921–926.

[33] P. Patil, S. Sehlangia, A. Patil, C. Pradeep, S.K. Sahoo, U. Patil, A new phthalimide based chemosensor for selective spectrophotometric detection of Cu (II) from aqueous medium, Spectrochim. Acta, Part A 220 (2019) 117129.

[34] P. Patil, S. Sehlangia, N. Patil, U. Patil, Dibenzimidamide based colorimetric chemosensor for Hg 2+ ions- A new application of famous drug, Res. J. Chem. Environ. 23 (10) (2019) 67–75.

[35] N.B. Patil, U.D. Patil, P.A. Patil, S. Bothra, S.K. Sahoo, S. Sehlangia, C.P. Pradeep, A.A. Patil, S.R. Patil, A fused benzothiazolo-pyrimidine-based chemosensor for selective optical detection of Fe 3+ and I ions in aqueous media, ChemistrySelect 4 (2019) 4185–4189.

[36] N. Kshirsagar, R. Sonawane, P. Patil, J. Nandre, P. Sultan, S. Sehlangia, C.P. Pradeep, Y. Wang, L. Chen, S.K. Sahoo, Fluorescent chemosensor for Al(III) based on chelation-induced fluorescence enhancement and its application in live cells imaging, Inorg. Chim. Acta. 511 (2020) 119805.

[37] S.R. Patil, J.P. Nandre, P.A. Patil, S. Bothra, S.K. Sahoo, A. Klasek, J.R. López, P.P. Mahulikar, U.D. Patil, Quinolone based chemosensor for the naked-eye and spectrophotometric detection of Cu2+ in aqueous media, Inorg. Chem. Commun. 49 (2014) 59–62.

[38] U.D. Patil, P.P. Mahulikar, A convenient, TiCl4/SnCl4-mediated synthesis of N-phenyl or N-aryl benzamidines and N-phenylpicolinamidines, ISRN Org.Chem., 2012. Article ID 963195.

[39] P. Job, formation and stability of inorganic complexes in solution, Ann. Chim. Appl. 9 (1928) 113–203.

[40] H.A. Benesi, J.H. Hildebrand, A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons, J. Am. Chem. Soc. 71 (1949) 2703–2707.

[41] A.H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.1, Gaussian, Inc., Wallingford CT, 2009.