A uracil nitroso amine based colorimetric sensor for the detection of Cu$^{2+}$ ions from aqueous environment and its practical applications†

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A simple uracil nitroso amine based colorimetric chemosensor (UNA-1) has been synthesized and screened for its cation recognition ability. Sensor UNA-1 exhibited a high sensitivity and selectivity towards Cu$^{2+}$ ions in aqueous medium in the presence of a wide range of other competing cations (Ag$^+$, Al$^{3+}$, Ba$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Cr$^{3+}$, Cs$^+$, Fe$^{2+}$, Fe$^{3+}$, Li$^+$, Mg$^{2+}$, Mn$^{2+}$, Na$^+$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Hg$^{2+}$ and Sr$^{2+}$). With Cu$^{2+}$, the sensor UNA-1 gave a distinct color change from colorless to dark yellow by forming a complex of 1 : 1 stoichiometry. Furthermore, sensor UNA-1 was successfully utilized in the preparation of test strips and supported silica for the detection of Cu$^{2+}$ ions from aqueous environment.

Introduction

Copper, the third-most abundant transition element in the body, plays an important role in various physiological processes such as hemoglobin biosynthesis, bone development, dopamine production, nerve function regulation, gene expression, and the functional and structural enhancement of proteins.1–6 Due to its redox-active nature, copper serves as an essential co-factor for a variety of metalloenzymes in living organisms such as cytochrome c oxidase, lysyl oxidase, copper–zinc superoxide dismutase and tyrosinase, which have a significant role in the enzymatic defense against oxygen toxicity.7–9 However, at high concentration, its redox properties turn into biologically hazardous materials because of its ability to generate reactive species, which create problems in the cellular metabolism.6–9 Apart from the biological and environmental importance, other advantages are that copper is relatively abundant, of low cost, and possess good malleability, electrical conductivity, thermal conductivity, chemical stability as well as germicidal efficiency.

All of these properties make copper central to the pharmaceutical and industrial sectors for making alloys, electrical wires, machine parts, batteries, drugs and fertilizers etc.10,11 However, with excessive loading, Cu$^{2+}$ is highly toxic to living organisms. For example, its over accumulation in human being leads to various diseases including neurodegenerative diseases such as Alzheimer's disease, Wilson's disease, Menkes disease, prion disease, gastrointestinal disorders, kidney damage, amyotrophic sclerosis, lipid metabolism and inflammatory disorders.12–15

The World Health Organization (WHO) have reported that the maximum limit of copper in drinking water should be 2 ppm (30 μM).16 Under normal conditions, the average concentration of copper in the blood should not exceed 100–150 μg dL$^{-1}$.17 However, due to the widespread use of copper in household appliances, industry, agricultural and water-pipes, Cu$^{2+}$ pollution has increased immensely throughout the world. Therefore, it is necessary to develop fast, convenient and reliable analytical methods for the qualitative and quantitative detection of copper, particularly in drinking water and in biological samples. Several analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectroscopy (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and voltammetry, quantum-dot-based assay have been developed for the qualitative and quantitative detection of Cu$^{2+}$ ions at trace levels. These technologies can detect Cu$^{2+}$ ion selectively with high sensitivity, but tend to need highly sophisticated and expensive instrumentation, and require tedious sample preparation and highly trained operators.18–21 By contrast, naked-eye detection methods permit detection of the target analyte at the micro/submicromolar levels without any need for...
expensive/sophisticated instrumentation.22,23 Therefore, given the importance of and the hazardous roles played by copper, we were encouraged to develop a colorimetric sensor with naked-eye capability for detecting Cu²⁺ from pure aqueous media.

On surveying the literature, we have noted that most of the reported Cu²⁺ selective colorimetric sensors have a number of drawbacks, viz. long response times, poor detection limits, tedious synthetic procedures, use of organic solvents, and interference from other transition metal ions (Table S1†).23–32 Herein, as a part of our ongoing research on chemosensors,23–45 we have developed a simple and easy to prepare colorimetric chemosensor, namely the uracil nitroso amine derivative UNA-1 (Scheme 1), which can be used for the highly selective and sensitive recognition of Cu²⁺ ions. This chemosensor gives a visual color change from colorless to clear dark yellow allowing for the naked-eye detection of Cu²⁺.

Results and discussion

Naked-eye detection of Cu²⁺

The synthesis of the uracil nitroso amine derivative (UNA-1) was achieved in two steps by the reaction of ethyl-cyanoacetate and N-methylurea in the presence of sodium ethoxide as a catalyst under acidic medium to give pyrimidine 1, which further undergoes nitrosation with sodium nitrite to afford UNA-1 (Scheme 1). Then, the colorimetric sensing ability of UNA-1 (5 × 10⁻⁵ M, in CH₃OH–H₂O, 10 : 90, v/v) was tested via the addition of 5 equivalents of various cations (1 × 10⁻² M, in H₂O). As shown in Fig. 1, UNA-1 exhibits a selective, sensitive and qualitative recognition of Cu²⁺ ions in day light/sunlight through a distinct visual color change from colorless to dark yellow. Noticeable color change was observed in the presence of any other cations screened herein.

The concentration dependent naked-eye study was performed (Fig. S1a†) by addition of various concentrations of UNA-1 (A = 1 × 10⁻³ M, B = 1 × 10⁻⁴ M, C = 1 × 10⁻⁵ M, D = 5 × 10⁻⁶ M, E = 1 × 10⁻⁶ M, F = 5 × 10⁻⁶ M and G = 1 × 10⁻⁷ M) to a fixed concentration of Cu²⁺ ions (1 × 10⁻³ M, in H₂O). The observed color change clearly suggested that the sensor was quite sensitive up to a concentration of 5 × 10⁻⁵ M for the detection of Cu²⁺ ions. Next, we investigated the effect of changing the concentration of Cu²⁺ ions from 1 × 10⁻³ M to 1 × 10⁻⁷ M to a fixed concentration of UNA-1 (1 × 10⁻⁴ M), which inferred that our sensor was able to detect Cu²⁺ up to the concentration of 1 × 10⁻⁵ M (Fig. S1b†).

Cation sensing studies

The cation recognition behavior of sensor UNA-1 with group I, II and III metal ions (Ba²⁺, Ca²⁺, Cs⁺, Li⁺, Mg²⁺, Na⁺, Al³⁺ and Sr²⁺) and transition and heavy metal ions (Co³⁺, Cu²⁺, Cr³⁺, Fe³⁺, Cd²⁺, Fe²⁺, Mn²⁺, Ni²⁺, Pb²⁺, Zn²⁺ and Hg²⁺) was investigated using UV-Vis absorption spectroscopy. The absorption spectrum of sensor UNA-1 in CH₃OH–H₂O (10 : 90, v/v) solvent system exhibited two absorption bands centered at 227 nm and 315 nm due to π-π* and n-π* electronic transitions, respectively. Upon addition of 5 equivalents of different cations (50 μL, 1 × 10⁻² M, in water) to a 5 × 10⁻⁵ M solution of UNA-1 in CH₃OH–H₂O (10 : 90, v/v), only the Cu²⁺ ions was able to perturb the absorption spectrum of UNA-1 effectively. The addition of aqueous Cu²⁺ ions to the UNA-1 solution led to the disappearance of the absorption band at 315 nm and the appearance of a new broad band between 335–500 nm due to the interaction of the paramagnetic Cu²⁺ with UNA-1 (Fig. 2). The appearance of a new charge transfer band was responsible for the naked-eye detectable color change of UNA-1.

The UV-Vis absorption titration was next performed upon successive addition of 1–10 equivalents of Cu²⁺ ions to the solution of UNA-1 to determine the binding ability and the limit of detection. With the incremental addition of Cu²⁺, the absorbance at wavelength 315 nm decreased continuously with the appearance of the new broad peak between 335–500 nm (Fig. 3). The titration resulted in the formation of an isosbestic point at 300 nm, which suggested the formation of a complex between UNA-1 and copper ions in solution.

The association constant (Kₐ) was estimated graphically by plotting 1/ΔA against 1/[Cu²⁺] (Fig. 4). The data was linear (fitted according to the Benesi–Hilderbrand equation) and the Kₐ value was obtained from the slope and intercept of the line. The Kₐ value for the UNA-1 copper complex was found to be 2.8 × 10⁴ M⁻¹ (R² = 0.9933). The value suggested that the sensor UNA-1 has high affinity towards Cu²⁺ ions. The limit of detection (LOD) and limit of quantification (LOQ) of UNA-1 were also calculated from the absorption titration data. According to the IUPAC definition, the LOD and LOQ were calculated using the relationship LOD = (3.3 × standard deviation)/slope and LOQ = (10 × standard deviation)/slope. To calculate the relative standard deviation, the absorption measurements of ten blank samples were taken. As shown in Fig. S2,† the absorbance calibration values were normalized between the minimum intensity and the maximum intensity and then a linear regression curve was fitted to these normalized data to get the slope. With this approach, the LOD and LOQ were found to be 10 μM and 33 μM, respectively.

![Scheme 1] Synthesis of UNA-1
The 1:1 binding stoichiometry for the complexation between UNA-1 and Cu²⁺ was determined using a Job’s plot experiment (Fig. 5) and a mole ratio plot (Fig. 3, inset). Furthermore, more direct evidence for the formation of this 1:1 complex was obtained from the ESI-MS spectra of UNA-1 in 1.0 equivalent of Cu²⁺ in methanol-water (10:90, v/v) (Fig. S3†). For pure UNA-1, a characteristic peak at m/z = 207.0405 was obtained which corresponds to the species [(UNA-1)$\cdot$2H₂O + H]⁺. Upon addition of copper perchlorate, the peak at 207.0405 disappeared and a new peak appeared at m/z = 287.0051 corresponding to the species [(UNA-1)$\cdot$Cu$\cdot$3H₂O]$^+$ (Fig. S3†). MS-MS of 287.0051 peak corresponding to the hydrated copper complex of UNA-1 shows fragmentation giving peaks at 251.94 and 233.97 corresponding to the species [(UNA-1)$\cdot$Cu$\cdot$H₂O]$^+$ and [(UNA-1)$\cdot$Cu]$^+$ respectively (Fig. S3†).

The chemosensor UNA-1 can bind to the Cu²⁺ ion via binding sites consisting of an amino and a nitroso group. All the crystal structures reported for complexes with similar ligands show the coordination through amino nitrogen and nitrogen of nitroso group.36-41 Thus, the lone pair of electrons on the nitrogen atoms of the amino and nitroso groups of the sensor UNA-1 are delocalized to the vacant orbital localized on the Cu²⁺ as shown in the Fig. 6. This electron donation or charge transfer gave rise to a color change from colorless to clear yellow. The charge of the copper is +2 and hence there should be two negative charges in our proposed structure for charge neutrality. Therefore, we
propose the deprotonation of $\text{NH}_2$ group and the inclusion of ClO$_4^-$ counter ion in the complex formula. Further, for more evidence of the binding of the Cu$^{2+}$, we carried out $^1$H NMR titration studies on UNA-1 by adding Cu$^{2+}$ solutions (Fig. S4†). It was observed that the peak at $\delta$ 12.91 corresponding to the $\text{NH}_2$ protons showed an up-field shift from 12.91 to 12.52 ppm accompanied by a broadening of the peak, while the peak at $\delta$ 9.08 corresponding to the $\text{NH}$ proton shows a small downfield shift from 9.08 to 9.28 ppm with broadening of the peak on addition of 1.0 equivalent of copper perchlorate. These observed shifts could be due to the complexation as proposed earlier. The possible 3D structure and the charge transfer processes occurring during the encapsulation of Cu$^{2+}$ by UNA-1 was investigated by density functional theory (DFT) calculations. The optimized structure of UNA-1 and its complex with Cu$^{2+}$ are shown in Fig. 7. On complexation of UNA-1 with Cu$^{2+}$, a lowering in the interaction energy by $-145.16$ kcal mol$^{-1}$ was observed, which indicates the formation of a stable complex.

The analytical applicability of UNA-1 was first tested by performing competitive experiments. The absorption and color changes caused by the mixture of Cu$^{2+}$ with the other metal ions was similar to that caused by Cu$^{2+}$ alone (Fig. S5†), which indicates that the other metal ions did not interfere with the binding of the chemosensor UNA-1 with Cu$^{2+}$. Secondly, the reversibility of UNA-1 for the detection of Cu$^{2+}$ was examined. To perform the reversibility test, a stock solution of UNA-1 (1 $\times$ 10$^{-3}$ M) was first treated with 1 equivalent of Cu$^{2+}$. The color of the solution changes from colorless to yellow. To the same solution, the reverse color change from yellow to colorless was observed upon addition of 4 equivalents of aqueous EDTA solution (Fig. S6 and ESI Video†). This result demonstrated the reversibility of the sensor UNA-1.

The sensing of Cu$^{2+}$ by UNA-1 worked very well on a solid support (Fig. 8 and ESI Video†). In this experiment, the silica gel (60–120 mesh, 10.0 g, colorless) was soaked with UNA-1 (in methanol, 50 mL, 1 $\times$ 10$^{-2}$ M) and then dried to afford a faint pink color silica gel due to the adsorption of the sensor on the surface. When the treated silica gel was added to a 10 mL aqueous solution of Cu$^{2+}$ (1 $\times$ 10$^{-3}$ M), the faint pink color promptly turned to a dark greenish/yellow color (ESI Video†). The instantaneous color change of the solid silica gel in aqueous solution clearly inferred the practical application of UNA-1 for the qualitative detection of Cu$^{2+}$ in aqueous medium. Then, the UNA-1 supported silica gel was treated with different concentrations of Cu$^{2+}$ ($B = 1 \times 10^{-3}$ M, $C = 1 \times 10^{-4}$ M, $D = 1 \times 10^{-5}$ M, $E = 1 \times 10^{-6}$ M), which indicated that the silica gel can be used to detect Cu$^{2+}$ up to 1 $\times$ 10$^{-5}$ M by a visually
detectable color change (Fig. 9). The results indicate that we can use this silica supported method not only in the determination of Cu²⁺ ions from water but also in the extraction/separation of Cu²⁺ ions from water.

In another approach, the practical utility of UNA-1 for the detection of Cu²⁺ was studied by developing a test paper strip. The cellulose paper (Whatman no. 42) was dipped in the methanolic solution of UNA-1 (1 × 10⁻² M) followed by drying in air to prepare the desire test strip. When this strip was dipped into an aqueous solution of Cu²⁺ (1 × 10⁻³ M), the colorless strip sharply turned to a yellow color (Fig. S7 and ESI Video†). The rapid color change of the test strip in solution clearly inferred the practical application of UNA-1 for the qualitative detection of Cu²⁺ in aqueous medium.

Conclusion

In summary, we have developed a simple uracil nitroso amine derivative UNA-1 for the colorimetric detection of Cu²⁺ in aqueous media. The sensor exhibited excellent selectivity and high sensitivity towards Cu²⁺ ions. The recognition of Cu²⁺ induced a clearly distinct color change of UNA-1 from colorless to yellow allowing for naked-eye detection. Moreover, UNA-1 can be applied to the detection of Cu²⁺ in aqueous media by a test paper strip and silica support method. These methods offer a very simple and quick detection for Cu²⁺ in aqueous media with a detection limit down to 10 μM. The good selectivity of sensor UNA-1 towards Cu²⁺ coupled with the use of pure water as the sole solvent in the detection process makes UNA-1 a promising candidate for the qualitative and quantitative detection of Cu²⁺ in various chemical, environmental and biological systems.

Experimental

Chemicals and instrumentation

Unless otherwise stated, all chemicals used for the synthesis of UNA-1 were of AR grade and were purchased either from S.D. Fine chemicals or Sigma Aldrich. All solvents were of spectroscopic grade and were used without further treatment. The aqueous stock solutions of cations (1 × 10⁻² M) such as Ag⁺, Al³⁺, Ba²⁺, Cd²⁺, Co²⁺, Cu²⁺, Cr³⁺, Fe²⁺, Fe³⁺, Mg²⁺, Mn²⁺, Ni²⁺, Pb²⁺, Zn²⁺, Na⁺, Sr²⁺ and Cs⁺ were prepared from their perchlorates salts; Ca²⁺, Hg²⁺ from its chloride salt and Li⁺ from its bromide salt. The stock solution of UNA-1 (1 × 10⁻³ M) was prepared in methanol and then diluted to 5 × 10⁻³ mol L⁻¹ with methanol-water (10:90, v/v).

The ¹H and ¹³C NMR spectra were recorded on a Jeol JNM-ECX 500 MHz multinuclear probe NMR spectrometer at ambient temperature in DMSO-d₆ with TMS as internal standard and chemical shifts reported in ppm. Mass spectra were recorded on a Bruker Compact HD mass spectrometer. The IR spectra were recorded on a Perkin Elmer FTIR spectrophotometer by using KBr discs and the IR bands are expressed in frequency (cm⁻¹). Absorption spectra were recorded on a Perkin Elmer U 3900 Co, USA UV/visible double beam spectrophotometer. The purity of the compound and progress of the reaction was monitored by means of a thin layer chromatography (TLC). Pre-coated silica gel 60 F₂₅₄ (Merck) on alumina plate (7 × 3 cm²) was used and visualized by using either an iodine or a short UV/visible lamp. Melting points were recorded on the Celsius scale by open capillary method and are uncorrected.

Synthesis of UNA-1

The chemosensor UNA-1 was synthesized by following the reported method in two steps as depicted in Scheme 1. In the first step, ethyl cyanoacetate (2.1 mL, 0.019 mol) was added from a dropping funnel under vigorous stirring to a solution of sodium ethoxide prepared from 0.92 g of metallic sodium and 20 mL of ethanol. A white solid was separated from the solution. After the complete addition of ethyl cyanoacetate, the mixture was stirred for 20 min at room temperature. N-Methylurea (1.20 g, 0.016 mol) was added and the mixture was heated for 3 h under reflux conditions on a water bath. The obtained white precipitate was filtered off, washed with ethanol, and then dissolved in 10 mL of water. The pH of the solution was adjusted to 7 by adding dilute sulphuric acid (2 N), and the mixture was stirred for 2 h to afford the pyrimidine 1 as product in 95% yield, m.p > 300 °C.

In the second step, a solution of 1.50 g of sodium nitrite in 4.0 mL of water was added to a mixture of 2.41 g of 5-amino-2,4-pyrimidine (1) and 12 mL of water. Then, 1.70 g of conc. H₂SO₄ was added dropwise under vigorous stirring. A solid precipitated, which was stirred for 6 h at room temperature, and the obtained product was filtered and washed with ethanol and water. Yield 86%. IR spectrum, ν, cm⁻¹: 3550, 3320, 3157, 3040, 2968, 2851, 1722, 1712, 1666, 1630, 1513, 1594, 1513, 1462, 1436, 1385, 1288, 1237, 1140, 1079, 1053, 512, 491. HRMS: m/z: 207.0405 corresponding to the species (UNA-1)⁻, 2H₂O + H. ¹H NMR (500 MHz, DMSO-d₆, δ ppm): 12.91 (s, 2H, −NH₂), 9.08 (s, 1H, −NH), 3.25 (s, 3H, −CH₃). ¹³C-NMR (125 MHz, DMSO-d₆, δ ppm): 27.95 (−CH₃), 139.23 (−CN=O), 146.25 (−CN=O), 149.50 (C=O), 160.40 (C=O) (Fig. S8–S11†).

Computational study

The structural optimization of UNA-1 and its host-guest complexes with Cu²⁺ was performed using the computer program Gaussian 09W by applying the density functional
theory (DFT) method. All the DFT calculations were performed in the gas phase with a hybrid functional B3LYP (Becke's three parameter hybrid functional using the LYP correlation functional) using the basis sets 6-31G (d,p) for C, H, N, O atoms and LANL2DZ for Cu atom.

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