LETTER

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A two-dimensional chromogenic sensor as well as fluorescence inverter: selective detection of copper(II) in aqueous medium†

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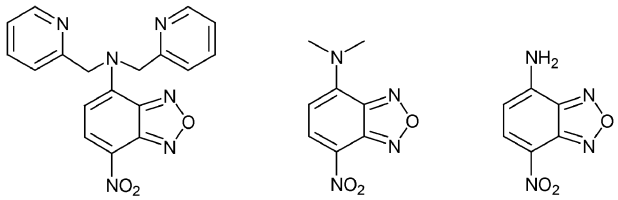
A new chromogenic sensor (1) has been synthesized that shows simultaneous blue shift and intensity loss of the absorption maximum as well as quenching of fluorescence, selectively, in the presence of Cu^{2+} ions at physiological pH.

Sensing and reporting of chemical species are currently of significant importance in chemistry, biology and environmental science.[1] In recent years, several luminescence-based devices for various analytes have been developed and studied for application in physiology and medical diagnostics.[1,2] The detection of Cu^{2+} has attracted attention,[3] as it participates in several bioenergetic and metabolic processes as well as in ecological pollution. Several fluorescent probes for the d^{10} metal ion Zn^{2+}, based on polypeptide, protein, macrocyclic and acyclic receptors have been designed in recent years.[4] However, development of practical fluorescent chemosensors for transition metal ions such as Cu^{2+} still remains a challenge[5] because of the inherent fluorescence quenching nature of the paramagnetic species. In most cases, to suppress the quenching interaction between these metal ions and the fluorophore, considerable synthetic effort had to be put in to obtain the sensor systems, usually employing a structurally well-developed receptor unit in the fluoroionophore system.[6] Chromogenic or colorimetric sensors are emerging as an attractive and alternative approach for the recognition of hazardous trace metals and will have applications in environmental and biomedical fields.[7] Recently, an azobenzene based colorimetric chemosensor for Cu^{2+} has been reported.[8] Even though the selectivity for Cu^{2+} is higher than for other metal ions, the similar changes at higher concentrations of Zn^{2+} and Cd^{2+} make this system essentially non-selective at different input levels.[8] Herein we describe a highly selective chromogenic sensor, 4-(di-(2-picolyl)amino)-7-nitrobenzoxa[1,3]diazole (**1**), which, in the presence of Cu^{2+} ions, shows two-dimensional changes in the absorption spectra (a blue shift of 42 nm and loss of absorption intensity) as well as quenching of fluorescence. Thus, we present a first example of a molecular *sensor* that shows large hypsochromic shift selectively in the presence of Cu^{2+} ions at physiological pH.



1 **2** **3**

Sensor **1**, constructed of ionophore and chromophore moieties, was easily synthesized through the reaction of 4-chloro-7-nitrobenzoxa[1,3]diazole and DPA (di-(2-picolyl)amine) in ethyl acetate. The desired product obtained, after purification by column chromatography (neutral alumina, hexane–EtOAc), was characterized using conventional methods. Compounds **2** and **3** were prepared according to the published procedure.[9]

In the absence of a cationic guest, the absorption spectrum of **1** is characterized by an intense band centered at 483 nm ($\varepsilon = 3.5 \times 10^4$ mol^{-1} L cm^{-1}) and a second band at 342 nm. The former is assigned to the intramolecular charge-transfer (ICT) transition because of the push–pull effect of the electron-donating amine and the electron-withdrawing nitro group. The spectral properties of **1** are dependent on pH between pH 1 and pH 5. Fig. 1 shows the changes in the absorption spectra upon titration of an alkaline solution of **1** (pH 13.5) with diluted HCl solution. The spectrum remains unaffected above pH 5. From pH 5 to pH 3, a gradual decrease in the intensity with a blue shift (17 nm) and an isosbestic point at

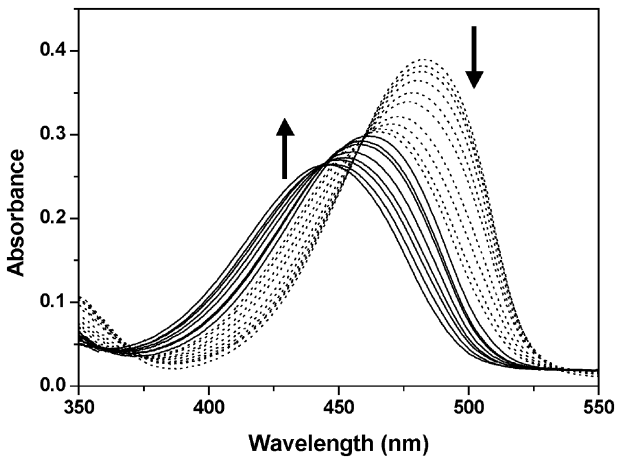


Fig. 1 Changes in the absorption spectra of an alkaline solution of **1** upon pH titration with acid; from pH 13.5 to pH 5.0 (no change); from pH 5.0 to pH 3.0 (dotted line); from pH 3.0 to pH 1.2 (solid line).

† Electronic supplementary information (ESI) available: absorption spectra depicting the reversibility of **1** and absorption spectra of **1** in the presence of other metal ions. See http://dx.doi.org/10.1039/b504823k

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460 nm was observed, corresponding to the protonation of the pyridyl moiety. Upon lowering of pH the spectrum is further blue-shifted (22 nm) with a new isosbestic point at 445 nm. Interestingly, the absorption spectrum of either **2** or **3** is independent of pH indicating that the amino nitrogen atom in 4-amino-7-nitrobenzoxa[1,3]diazole chromophores has no affinity for protons. This is not surprising because of strong electron withdrawing nitro group in conjugation. Thus, the observed blue shift of the absorption maximum of **1** in the presence of protons is attributed primarily to a redistribution of electron density due to the interaction between the protonated pyridyl moieties and the nitrogen atom connected to the chromophore. From these absorbance changes, pK_a values of 3.6 and 2.7 for the protonation of pyridyl moieties are evaluated.[10] The lower pK_a values than those reported for pyridine[11] are due to the electron-withdrawing tendency of the chromophore unit, which lessens the electron density at the pyridyl nitrogen atom thereby reducing its affinity for protonation. No changes in the absorption spectrum of **1** above pH 5 clearly demonstrate the utility of the sensor system in the physiological environment where pH $>$ 5.

The effect of metal ions on the absorption spectrum of **1** was examined in an aqueous solution of 10 mM HEPES buffer (pH 7.4). In the presence of alkali and alkaline earth metal ions such as Na^+, K^+, Mg^{2+} and Ca^{2+}, **1** does not show any obvious spectral change. This is not unexpected considering the available binding sites for complexation. Among transition metal ions, Cu^{2+} induces a drastic change in the UV-vis spectrum. As can be seen in Fig. 2, upon progressive addition of Cu^{2+}, the absorption gradually loses intensity with a blue shift from 483 nm to 441 nm. The observation of an isosbestic point at 450 nm indicates that this optical response arises from the presence of two species in equilibrium, **1** and **1**–Cu^{2+}.

It is expected that the electron density at the 4-N atom is reduced by the electron-withdrawing effect of coordinated metal ions thereby decreasing the charge separation in the electron donor–acceptor chromophore and that remarkable changes in the absorption spectra should be caused when complexed to a metal ion. As stated earlier, due to the unavailability of lone-pair of electrons for complexation, the 4-amino nitrogen atom could not be involved in coordination with the metal ions. Therefore, the binding sites for complexation with the metal ions are only the nitrogen atoms of the pyridyl moieties. However, as commonly believed, if only the pyridyl nitrogen atoms are involved in coordination with the Cu^{2+} ions, the energy states of the chromophore are not expected to be much affected as the pyridyl moieties are connected to the chromophore by a non-conjugating spacer

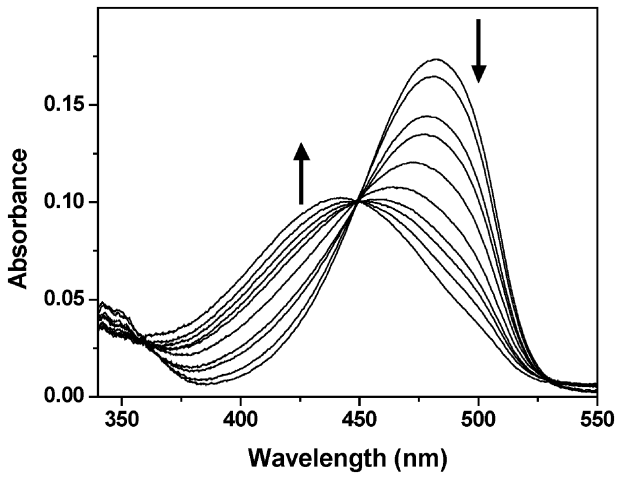

Fig. 2 Absorption spectra of **1** (5 μM) in water (pH 7.4, 10 mM HEPES) after the addition of 0, 0.05, 0.2, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2 mM Cu(II).

Table 1 PM3 calculated geometrical parameters for **1**, **1**–H^+ and **1**–$2H^+$

Geometrical parameters	**1**	**1**–H^+	**1**–$2H^+$
4-N–C (Å)	1.395	1.452	1.455
Angles around 4-N (°)	123.44	117.06	114.53
	118.88	115.18	120.30
	115.19	114.19	115.04
Formal charge on 4-N	+0.148	−0.007	−0.008
Formal charge on pyridyl N atoms	−0.094	+0.57	+0.54
	−0.069	−0.104	+0.55
Formal charge on nitro O atoms	−0.632	−0.568	−0.547
	−0.582	−0.555	−0.531

and there is no evidence of through-bond transfer of charge. Of course, a small hypsochromic shift due to the electrostatic interaction between the metal ion and the nitrogen atom associated with the chromophore moiety is known.[12] This suggests that upon complexation the electron density of the system is considerably redistributed giving rise to such a large blue shift, nevertheless, steric crowding upon coordination with the Cu^{2+} ions could also possibly cause a twisting of the 4-N–aromatic C bond leading to the blue shift. Thus, we show that while direct coordination of the metal ions by the nitrogen atom involved in the ICT process leads to the disappearance of the ICT band,[8] a non-bonding interaction, as in the present case, results in recognition of the guest by a hypsochromic shift of the absorption maximum.[12]

It has to be noted that the recognition of Cu^{2+} is found to be reversible as the addition of EDTA reverses the absorption spectra (see ESI†). Thus, **1** can be termed as a molecular *sensor*, a feature that requires reversibility and potential re-utilization. A similar system with a cyclam receptor at the 4-position, for the detection of Cu^{2+}, that acts as a molecular *dosimeter* (an irreversible device) is reported.[13] The irreversibility of the device limits its practical application.

A rational explanation for the observed blue shift of the absorption maximum of **1** in the presence of protons or Cu^{2+} ions may be obtained from molecular modeling and semi-empirical calculations.‡ Semi-empirical (PM3) calculations of **1** in the free, singly and doubly protonated forms gave results that indicate that upon protonation of the pyridyl moieties, the ICT character of the system is significantly altered (Table 1). The results also explain the lower than usual pK_a values obtained for the protonation of the pyridyl moieties.

The fluorescence spectrum of **1** in buffered water (pH = 7.4) is centered at 551 nm, which results from a charge transfer excited state. Addition of Cu^{2+} ions leads to a loss of fluorescence intensity with a very small hypsochromic shift (Fig. 3). Quenching of fluorescence is attributed to the electron or energy transfer between Cu^{2+} and the excited fluorophore.

Addition of transition metal ions other than Cu^{2+}, such as Ni^{2+} and Zn^{2+} does not result into any significant change in either the absorption or fluorescence spectrum of **1**. Fig. 4 highlights the selectivity and sensitivity of the present sensor system. At very high concentrations of these metal ions, however, a gradual reduction in the intensity of the absorption maximum of **1** with a small hypsochromic shift (8–12 nm) is observed (see ESI†). Thus, sensor **1** has a poor affinity for metal ions other than Cu^{2+}. The binding constant for Cu^{2+} is determined[10] as log $\beta = 3.5$ suggesting that the interaction of Cu^{2+} itself is not very strong. This is not surprising because the effect of removing the interaction with the 4-amino donor will reduce the binding constant significantly. Hence, metal ions such as Ni^{2+} and Zn^{2+}, placed below Cu^{2+} in the Irving–Williams series, are expected to have negligible interaction.

‡ Molecular geometry was optimized by the PM3 method using commercially available HyperChem software.

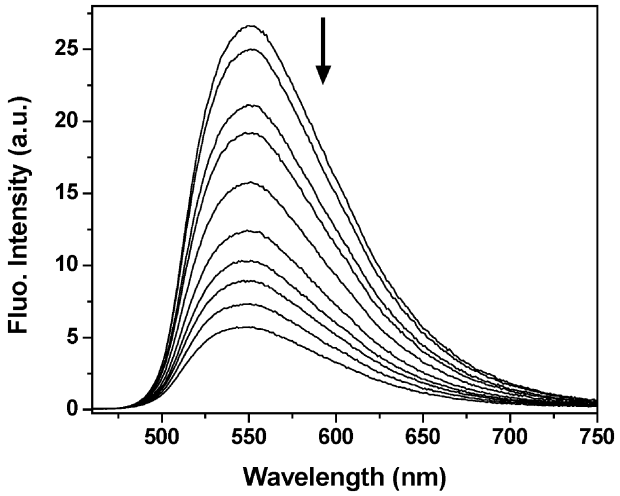

Fig. 3 Fluorescence spectra of **1** (5 μM) in water (pH 7.4, 10 mM HEPES) after the addition of 0, 0.05, 0.2, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2 mM Cu(II). $\lambda_{exc} = 450$ nm.

Comparatively, a very large blue shift of 42 nm only in the presence of Cu^{2+} ions makes the present system essentially selective for Cu^{2+} ions. More interestingly, the linearity in the absorption maximum of **1** *vs.* Cu^{2+} concentration profile (Fig. 4) over a certain range of concentrations makes the present system appropriate for the analytical detection of Cu^{2+} in solution. A three-dimensional plot shown in Fig. 5 represents the two-dimensional operation of **1**.

In summary, we have developed a simple chromogenic sensor **1** for selective detection of Cu^{2+} ions under physiological conditions (pH $>$ 5). This system also behaves as a fluorescence inverter. The exclusive two-parameter optical signaling of Cu^{2+} based on (i) a linear 483 nm-to-441 nm blue shift and (ii) the loss of absorbance intensity makes the present system a potential candidate for the detection of Cu^{2+} ions.

Experimental

Methods

The absorption and fluorescence spectra were recorded on a Shimadzu spectrophotometer (UV-3101PC) and Spex spectrofluorimeter (Fluorolog-3), respectively. NMR spectra were recorded on Bruker AVANCE 400 MHz spectrometer at ambient temperature and were referenced to the internal ^1H solvent peaks.

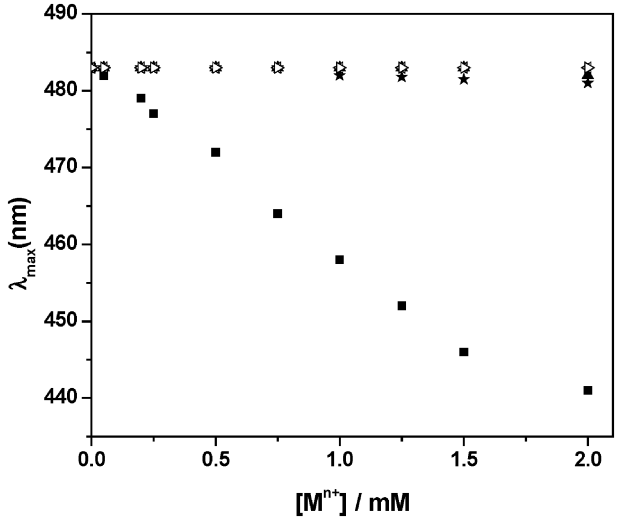

Fig. 4 Hypsochromic shift of the ICT absorption maximum of **1** induced by various metal cations; (■) Cu^{2+}, (★) Ni^{2+}, (▲) Zn^{2+}, (◁) Na^+, (▷) Mg^{2+}. [**1**] = 5×10^{-6} M.

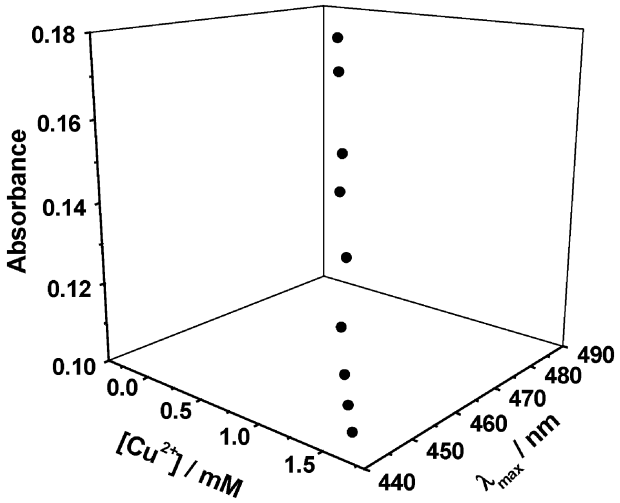

Fig. 5 A three-dimensional plot representing the two-dimensional operation of **1**.

Synthesis of 4-(di-(2-picolyl)amino)-7-nitrobenzoxa[1,3]diazole (1)

Di-(2-picolyl)amine (0.45 mL, 2.5 mmol) in ethyl acetate (30 mL) were placed in a 100 mL two-necked round-bottom flask and cooled to 0 °C. 4-Chloro-7-nitrobenzoxa[1,3]diazole (0.2 g, 1 mmol) dissolved in ethyl acetate (20 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h. The reaction was further carried out at room temperature for another 2 h to ensure completion. Subsequently, the solvent was evaporated under vacuum and the residue was purified by column chromatography (neutral alumina, hexane–ethyl acetate). Yield 82%. CHN analysis calculated for $C_{18}H_{14}N_6O_3$: C, 59.67; H, 3.87; N, 23.20. Found: C, 59.39; H, 3.61; N, 22.92. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.42 (bs, 4H), 6.33 (d, 1H), 7.23 (m, 4H), 7.67 (m, 2H), 8.37 (d, 1H), 8.56 (s, 2H).

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