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Ratiometric fluorescent detection of Cu(II) in semi-aqueous solution using a two-fluorophore approach

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ABSTRACT

Schiff base sensor **1**, containing naphthalene and naphthalimide fluorophores with separate and distinct emission wavelengths, showed good selectivity for Cu(II) over other tested physiological and environmentally important cations through changes in its fluorescence spectra in THF/H_2O (9:1) HEPES buffered solution. By taking the ratiometric change of the emissions at 435 nm (naphthalene–Schiff base) and 510 nm (naphthalimide) good linearity was observed in the 0–10 μM range. The enhancement of the 435 nm emission upon binding Cu^{2+} was attributed to a prevention of the rapid C=N isomerisation that otherwise leads to non-radiative decay, while the quenching of the naphthalimide emission was attributed to electron transfer between the excited naphthalimide fluorophore and the redox active Cu^{2+}.

Copper is an essential trace element required by many living organisms for normal physiological functioning. In humans, for example, copper-dependent enzymes are involved in critical biological processes such as mitochondrial electron transport and melanin production.[1] However, copper is also considered toxic if present in sufficient quantities. Due to its redox nature, copper can participate in reactions that result in the production of reactive oxygen species (ROS) responsible for deleterious cellular events such as lipid peroxidation and DNA damage.[2] Abnormal plasma concentrations of copper have also been found in patients undergoing renal dialysis and those suffering from leukaemia.[3] Therefore, the development of sensors for the accurate detection of copper remains an active area of research.

Fluorescence-based chemosensors remain popular due to their high sensitivity, adaptability and relative inexpense. Numerous examples of fluorescence sensors for Cu(II) are available in the literature.[4] However, the majority of these involve single wavelength measurements which can be problematic due to the errors associated with local sensor concentration, photobleaching and environmental factors.[5] These problems can be overcome by ratiometric analysis where the ratio of intensities at two wavelengths is taken at various concentrations of analyte.[6] Normally, this is achieved using molecules designed such that the binding site for the analyte is directly integrated with the reporting fluorophore, that is, their π-orbitals overlap.[7] As a result, an electronic redistribution of charge occurs in the excited state so that the unbound and bound forms of the sensor deposit their emission at spectrally distinct wavelengths.

An alternative, more simplistic approach was developed by Lippard and co-workers for the ratiometric determination of Zn^{2+} in HeLa cells.[8] It involved using two fluorophores, the first a fluorescein unit connected to dipicolylamine receptors via a methylene spacer. The second fluorophore, a coumarin unit, was connected to the fluorescein via an ester bond. This ester bond was cleaved by endogenous esterase enzymes when the sensor was taken up by HeLa cells distancing the two fluorophores from each other and eradicating any possible communication between the two. Thus, the emission of coumarin was unaffected by the intracellular Zn^{2+} concentration while the fluorescein emission was affected. The ratio of the two emissions gave an indication of the intracellular Zn^{2+} levels.

Here, we adopt a similar two-fluorophore approach for the ratiometric detection of Cu^{2+}. Compound **1** was designed to include a naphthalene-containing Schiff base receptor joined to a naphthalimide unit via an ethylene spacer (Scheme 1). It was synthesised by first reacting 4-bromo-1,8-naphthalic anhydride with allylamine to form **2**, which was then reacted with ethylene diamine to yield **3**, following known procedures.[9] Target compound **1** was isolated as a yellow solid following the condensation reaction of **3** with 2-hydroxy-1-naphthaldehyde.[10]

The photophysical properties of **1** were investigated in THF/H_2O (9:1, v/v) HEPES buffer solution (pH 7.0 ± 0.1). The UV–vis spectrum, shown in Figure 1, displayed three main bands with λ_{max} 310, 405 and 432 nm. The 310 and 405 nm bands are consistent with compounds containing the naphthalene-Schiff base structural motif[11] while the 432 nm band corresponds to the naphthalimide absorbance.[9]

The emission spectrum of **1** when excited at 350 nm is shown in Figure 2a which again shows two main bands with λ_{max} 435 and

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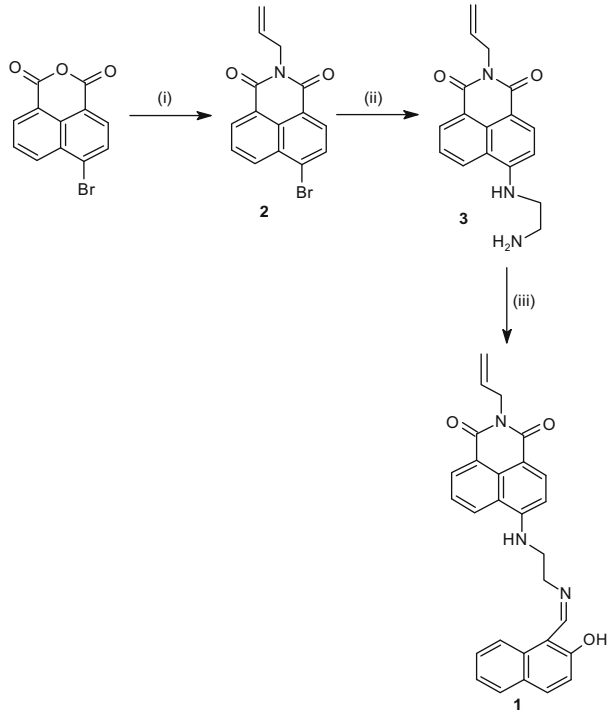

Scheme 1. Reagents and conditions: (i) allyl amine, DMF, 50 °C, 18 h, 82%; (ii) ethylene diamine, 80 °C, 18 h, 78%; (iii) 2-hydroxy-1-naphthaldehyde, dry MeOH, 25 °C, 18 h, 33%.

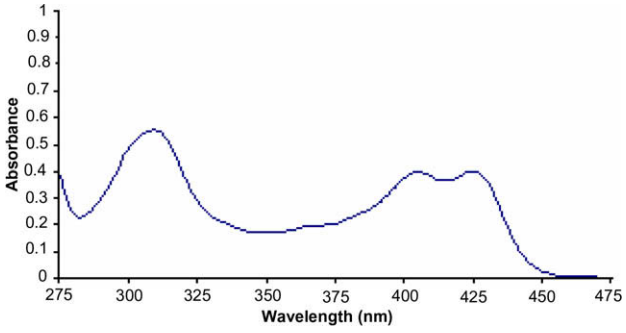

Figure 1. UV–vis spectrum of **1** in THF/H_2O (9:1, v/v) HEPES buffer solution (pH 7.0 ± 0.1). [**1**] = 20 μM.

510 nm, with the former also displaying a shoulder at λ_{max} 390 nm. The λ_{max} 435 nm band is consistent with emission from the *keto* tautomer of the naphthalene–Schiff base component caused by an excited state intramolecular proton transfer (ESIPT) process[11] while the latter can be attributed to emission from the naphthalimide unit.[9] The presence of emission from both the naphthalene and naphthalimide fluorophores discounts the likelihood of energy transfer occurring between the two despite a good spectral overlap between the naphthalene emission and naphthalimide absorbance.

The cation recognition behaviour of **1** was evaluated from changes in fluorescence intensity by addition of a particular metal salt (50 μM) to a solution of **1** (20 μM). Addition of Mg^{2+}, Fe^{3+} and Mn^{2+} resulted in small quenches of both emission bands at λ_{max} 435 nm and λ_{max} 510 nm. In contrast, addition of Cu^{2+} resulted in an enhancement of the λ_{max} 435 nm band accompanied by a significant quenching of the λ_{max} 510 nm band. Addition of Li^+, Na^+, K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} ions caused only minor changes in the fluorescence spectrum of **1** under the same conditions. When the ratiometric change in the intensity of the 435 and

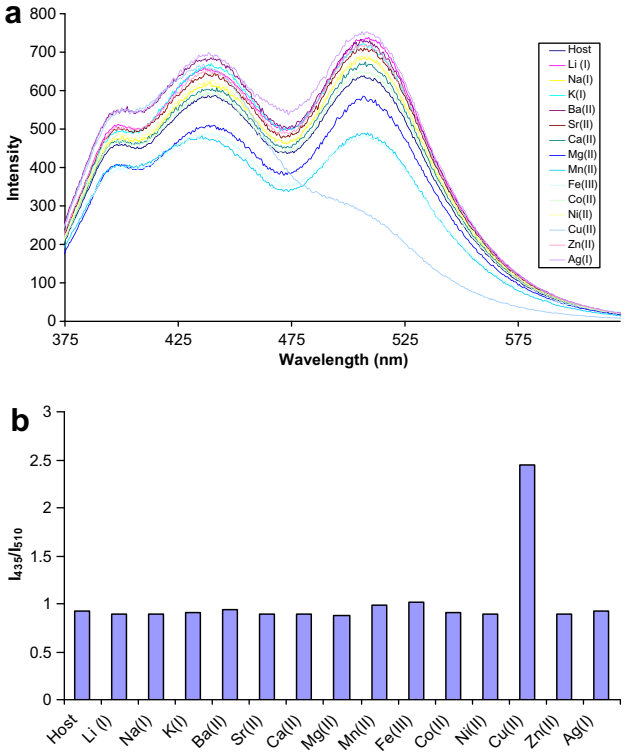

Figure 2. (a) Fluorescence spectra of **1** in the presence of various metal ions and (b) bar graph showing the ratiometric change (I_{435}/I_{510}) observed for **1** against each of the tested ions. [**1**] = 20 μM, [ion] = 50 μM, THF/H_2O (9:1, v/v) HEPES buffer solution (pH 7.0 ± 0.1).

510 nm bands (I_{435}/I_{510}) was determined for each ion (Fig. 2b) excellent selectivity was observed for Cu^{2+} over the other tested cations. This was not totally unexpected due to the position of copper in the Irving Williams series, meaning it strongly binds to ligands irrespective of their nature or number.[12]

Figure 3a shows the effect of the continuous addition of Cu^{2+} ions on the fluorescence spectrum of **1**. The results show that upon increasing the amount of Cu^{2+}, a gradual increase in the intensity of the 435 nm band was accompanied with a gradual decrease of the 510 nm band.

When the ratiometric intensity change (I_{435}/I_{510}) was plotted as a function of Cu^{2+} concentration, good linearity was observed in the 0–10 μM range (Fig. 3b).

The reason for the increase in the emission intensity of the 435 nm band, attributed to naphthalene–Schiff base emission, is most likely due to a prevention of the rapid C=N bond isomerisation upon Cu^{2+} binding that otherwise leads to non-radiative decay of the excited state.[13] The quenching of the 510 nm emission can be attributed to the redox active Cu^{2+} being brought into proximity of the naphthalimide fluorophore when it binds to the Schiff base region of **1** and quenches the excited state by electron/energy transfer.[14] We believe this Cu^{2+} quenching effect also reduces significantly the enhancement of the naphthalene fluorescence at 435 nm. In other words, the enhancement brought about from the prevention of C=N isomerisation is cancelled somewhat by a simultaneous quenching effect by Cu^{2+} on the naphthalene emission. We were unable to probe further the binding event between **1** and Cu^{2+} by NMR due to the paramagnetic nature of Cu^{2+} and, unfortunately, were unable to grow suitable crystals of the **1**:Cu^{2+} complex to determine the exact binding mechanism. However, the slight blue shift (8 nm) that accompanies the enhancement of the 435 nm band indicates that **1** binds Cu^{2+} in the Schiff

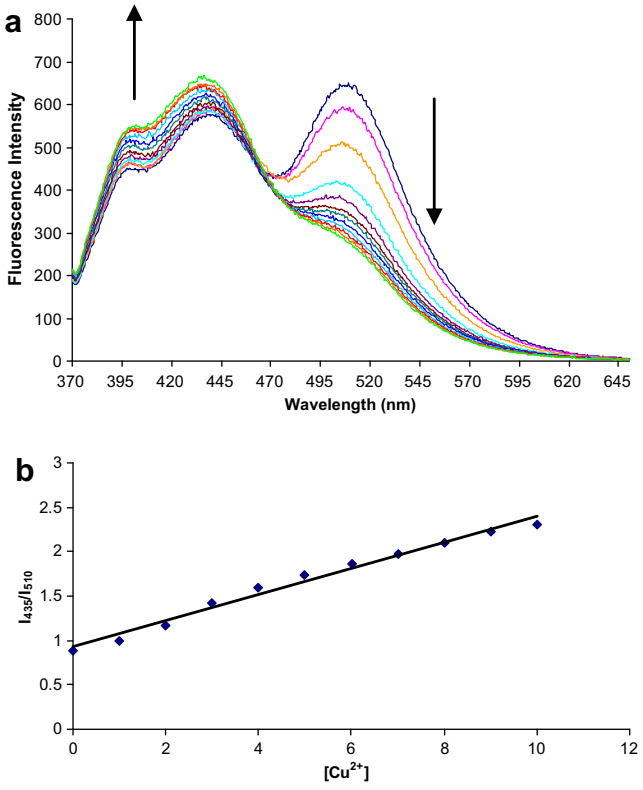

Figure 3. (a) Fluorescence spectra of **1** in the presence of various amounts of Cu^{2+} (0–10 μM), (b) linearity plot for **1** in the presence of Cu^{2+}. Solvent = THF/H_2O (9:1, v/v) HEPES buffer solution (pH 7.0 ± 0.1). [**1**] = 20 μM.

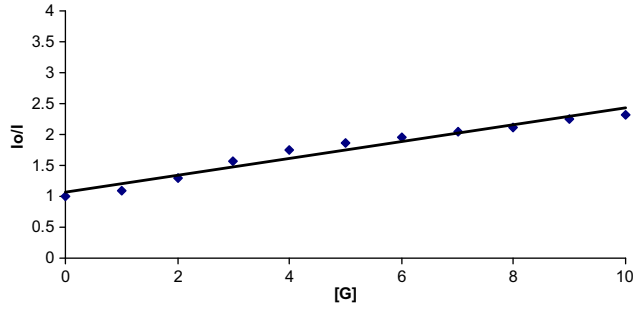

Figure 4. Stern Volmer plot for **1** in the presence of Cu^{2+}. Solvent = THF/H_2O (9:1, v/v) HEPES buffer solution (pH 7.0 ± 0.1).

base region without assistance of the aniline lone pair of the naphthalimide, as no wavelength shift was observed to accompany the quenching of the 510 nm band. The quenching of this band was further investigated using the Stern Volmer equation. When the equation was derived for a 1:1 host–guest complex an excellent fit was obtained (see Fig. 4), indicating this to be the most likely species formed between **1** and Cu^{2+}.

In summary, a two-fluorophore approach has been adopted for the ratiometric detection of Cu^{2+} in semi-aqueous solution buffered at pH 7.0. The sensor displays excellent selectivity for Cu^{2+} over a range of other cations and shows good linearity between 0 and 10 μM. We believe that this two-fluorophore approach offers an alternative method to the development of new ratiometric sensing systems.

Acknowledgements

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