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SHORT COMMUNICATION

A New Fluorescent Sensor for the Determination of Iron(III) in Semi-Aqueous Solution

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Abstract A simple fluorescent sensor **1** has been developed for the recognition of Fe(III) in semi-aqueous solution at pH 7.0. The sensor, containing two Schiff base type receptors directly connected to naphthalene fluorophores, shows a concentration dependent decrease in emission intensity upon Fe(III) addition. The sensor was selective for Fe(III) over other metal ions and can measure Fe(III) ion concentration between 0.05 and 0.12 mM. The binding stoichiometry was established as 1:1 (host: guest) with a binding constant (Logβ) of 4.01. Furthermore, the addition of Fe(III) to a solution of **1** caused a colour change from light yellow to colourless meaning **1** is also capable of detecting Fe(III) by the naked eye.

Keywords Schiff base · Fluorescence · Sensor · Iron(III)

Introduction

Iron plays a crucial role in a variety of vital cell functions such as oxygen metabolism and electron transfer processes in DNA and RNA synthesis. Most of the iron present in biological systems is tightly associated with enzymes and specialized transport and storage proteins [1, 2]. However, a minor fraction of iron (generally called 'labile iron') is bound comparatively loosely to a heterogeneous population of organic anions (phosphates and carboxylates), polyfunctional ligands (i.e. chelates, siderophores, and polypeptides), surface components of membranes (e.g., phospholipid head groups), or extracellular matrix (e.g., glycans and sulfonates) [3]. In the presence of molecular oxygen, labile iron is able to redox cycle between the two most stable oxidation states Fe^{2+} and Fe^{3+}, and fosters the generation of highly destructive oxygen species such as hydroxyl radical via the Fenton reaction [4, 5]. These highly reactive radicals are capable of interacting with most types of biological material including sugars, lipids, proteins, and nucleic acids, resulting in peroxidative damage [6]. Since man lacks the effective means to protect human cells against iron overload, serious complications like β-thalassemia is largely achieved through the regulation of iron absorption [7]. Moreover, hereditary hemochromatosis is characterized by excess iron that causes tissue damage and fibrosis with irreversible damage to various organs. Furthermore, iron homeostatis is an important factor involved in neuroinflammation and progression of Alzheimer's disease [8]. Similarly, iron deficiency (hypoferremia) can be as harmful, or even more harmful in certain conditions than iron overload. Therefore, the ability to detect Fe(III) in a selective and accurate manner is of high importance.

Fluorescence based sensing protocols provide significant benefits over other detection methods due to their high sensitivity, rapid response rate and relative inexpense. In addition, fluorescence sensors can be easily incorporated in portable diagnostics as evidenced by the OPTI critical care analyzer developed for the determination of blood electro-

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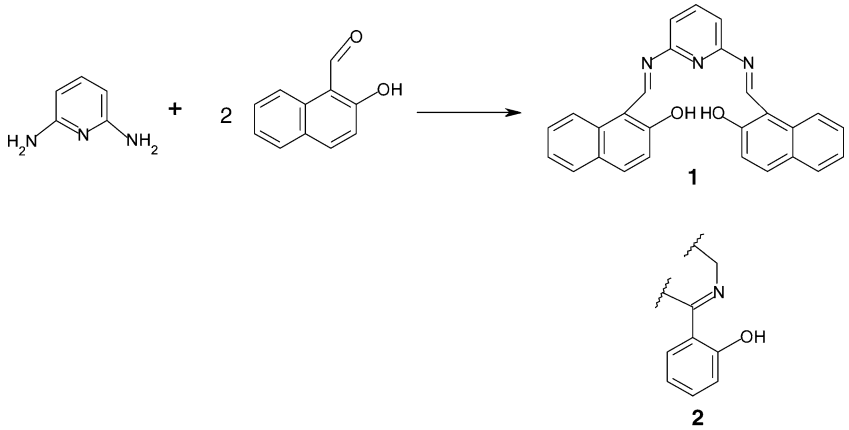
Scheme 1 Synthesis of probe **1**



lytes [9]. Not surprisingly, therefore, there has been significant interest in the development of new fluorescence sensors for a range of different ions including Fe(III). In this manuscript, we present **1** as a novel fluorescent sensor capable of determining Fe(III) concentrations in semi-aqueous solution by changes in its fluorescent intensity.

Materials and Methods

All reagents used were of the highest grade obtainable and were purchased from Aldrich. Absorbance measurements were recorded on a Varian Cary 50 Spectrometer using 10 mm quartz cuvettes. Fluorescence measurements were recorded on a Cary Eclipse fluorimeter using 10 mm quartz cuvettes. Excitation slit size was 10 nm and emission slit size was 10 nm. NMR spectra were recorded on a Varian 500 MHz spectrometer. ESI-MS spectra were obtaind using a LCQ^{TM} quadrupole ion-trap mass spectrometer (Finnigan MAT, San Jose, California, USA) utilising electrospray ionisation (ESI).

Synthesis of **1**

2-hydroxy-1-naphthaldehyde (1.66 g, 9.65 mmol) was added to 2,6-diaminopyridine (0.5 g, 4.58 mmol) in dimethylformamide (15 mL) at room temperature. After stirring over night, the solvent was evaporated under reduced pressure and the crude product dissolved in hot ethanol and filtered. Upon cooling an orange coloured product was obtained and dried *in vacuo* to yield 1.24 g of **1** (65% yield). ^{1}H NMR, 500 MHz (DMSO): 6.96 (2H, d, Pyr-H), 7.05 (2H, d, Naph-H), 7.38 (2H, dd, Naph-H), 7.59 (2H, dd, Naph-H), 7.68 (2H, d, Naph-H), 7.82 (3H, m, Pyr-H_4, Naph-H), 8.19 (2H, d, Naph-H), 10.04 (2H, d, CH=N). ^{13}C NMR, 125 MHz (DMSO): 112.9, 119.2, 122.5, 124.7, 128.0, 129.3, 129.7, 132.2, 138.8, 164.3, 193.4 I.R. ν_{max} (cm^{-1}) 2,922, 2,852, 1,628, 1,534, 1,458, 1,290, 1,152, 736.

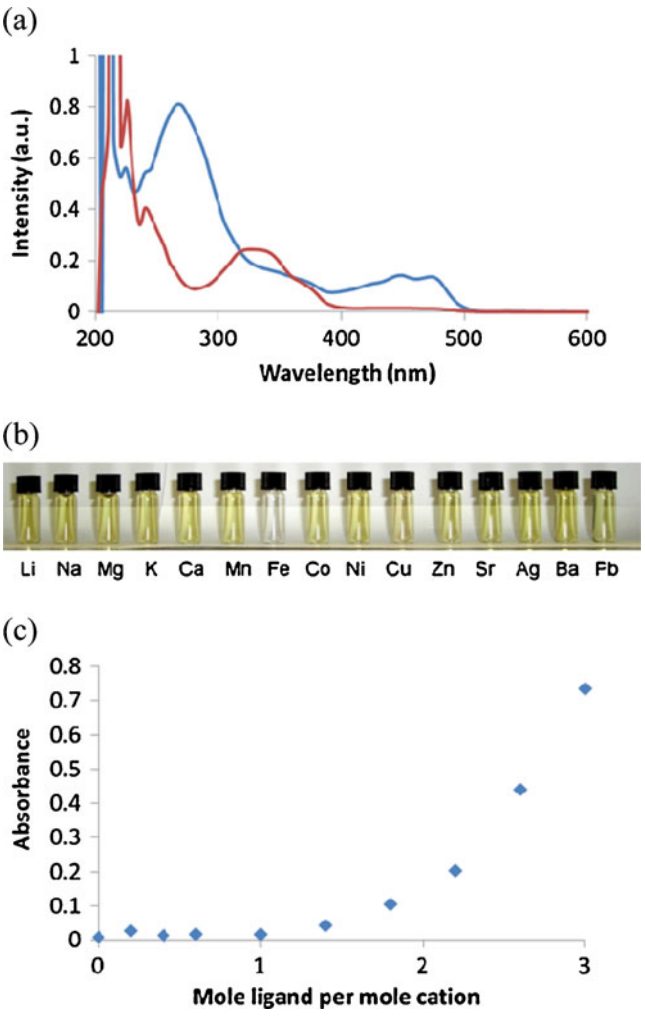


Fig. 1 **a** UV–vis spectra of **1** in the absence (*blue line*) and presence of 50 μM Fe(III) **b** photograph of vials containing **1** in the presence of various metal ions with a distinct colour change from yellow to colourless being observed for Fe(III) **c** plot of absorbance at 475 nm against mole ligand per mole Fe(III) where the amount of Fe (III) was kept constant and the amount of **1** gradually increased. Solvent: THF: H_2O (9:1,v/v) HEPES buffer solution (pH=7.0±0.1). [**1**]=10 μM, [metal salt]=50 μM for **b**

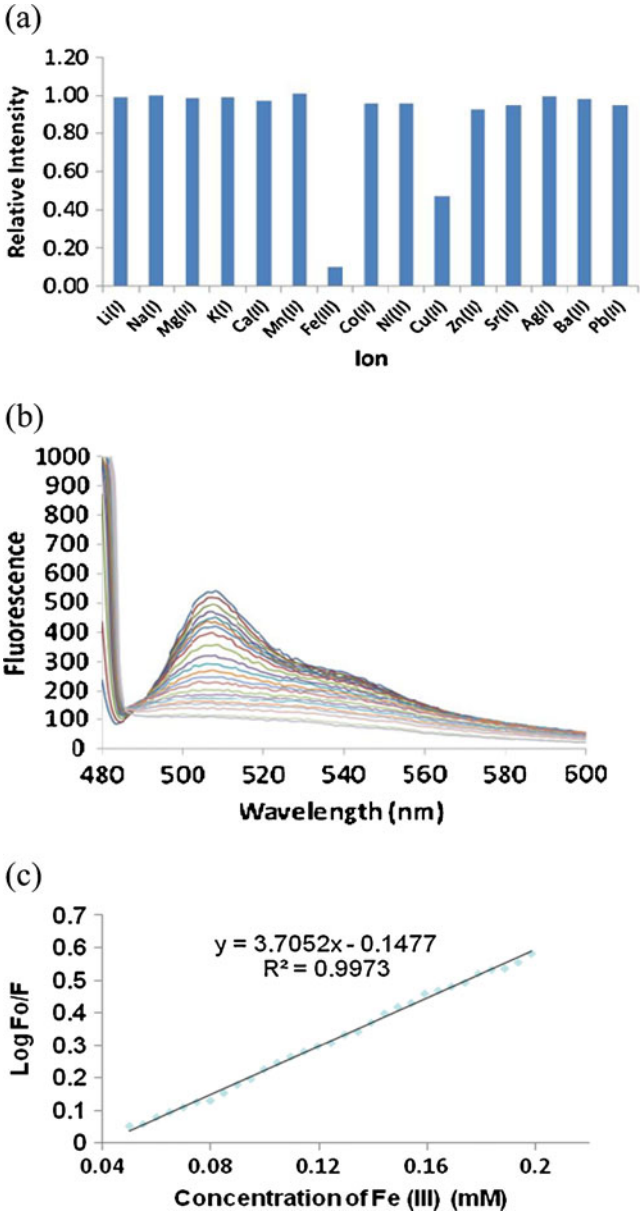


Fig. 2 **a** Bar chart illustrating the selectivity of **1** agaisnt a range of metal ions **b** fluorescence spectra of **1** upon increasing Fe(III) addition. **c** Stern-Volmer plot of Log F_0/F (starting intensity/actual intensity) against Fe (III) concentration. for **1**. Solvent: THF:H_2O (9:1, v/v) HEPES buffer solution (pH=7.0±0.1). [**1**]=10 μM, [metal salt]= 50 μM for (**a**)

HRMS: Calculated for $C_{27}H_{19}N_3O_2$=417.1477; measured mass=417.1596.

Results and Discussion

Compound **1** was prepared in 65% yield by the facile reaction of 2-hydroxy-1-naphthaldehyde with 2,6-diaminopyridine in dimethylformamide (DMF) solvent (Scheme 1). Although the preparation of **1** has been reported before, its use as a

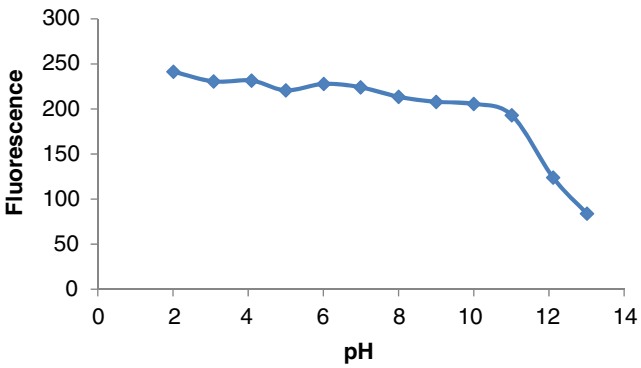


Fig. 3 Plot of fluorescence intensity against pH for **1**

fluorescent probe has not yet been investigated [10]. **1** contains two hydroxynaphthalene units connected via Schiff base units to a central pyridine scaffold. Thus, **1** contains binding units similar to siderophores, which are the iron chelating compounds secreted by microorganisms to facilitate the active transport of iron [11]. It has been demonstrated that many siderophores and iron chelating agents comprise a phenol substituent adjacent to a sp^2 hybridised nitrogen atom as depicted in structure **2** (Scheme 1) [12–14].

The UV–vis spectrum of **1**, recorded in a THF/Water (9:1) solvent system buffered at pH 7.0, exhibited two main bands at λ_{MAX} 280 and 475 nm, reflecting the aryl and naphthalene chromophores respectively (Fig. 1a). Significant changes in the absorption spectrum of **1** were observed upon the addition of Fe(III). As shown in Fig. 1a, a significant blue shift from 475 nm to 325 nm was observed upon addition of 50 μM Fe(III) to a 10 μM solution of **1**. This effect was so significant it could be observed by the naked eye with the solution changing from yellow to colorless (Fig. 1b). To determine the binding stoichiometry between **1** and Fe(III) the mole ratio method was employed monitoring absorbance at 475 nm [15]. As shown in Fig. 1c, the absorbance at 475 nm remained low until one molar equivalent of **1** was added, after which the absorbance increased significantly. This suggests as sensor is added to a fixed solution of Fe(III) it forms a **1**-Fe(III) 1:1 complex which gives rise to the

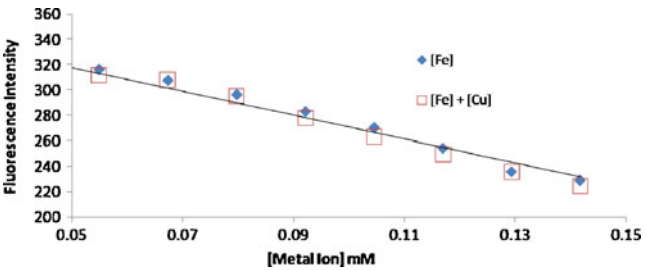


Fig. 4 Plot of fluorescence intensity a against metal ion concentrations for solutions containing equimolar concentration of metal ions. Solvent: THF:H_2O (9:1,v/v) HEPES buffer solution (pH=7.0± 0.1). [**1**]=10 μM

absorption band with λ_{MAX} 325 nm. However, after one molar equivalent of sensor has been added, the excess remains unbound in solution giving rise to the absorption band with 475 nm (i.e. the λ_{MAX} of **1**).

The fluorescence properties of **1** were then investigated again in a THF/Water (9:1) solvent system buffered at pH 7.0. When excited at 475 nm a broad fluorescence emission was observed with λ_{MAX}=507 nm (Fig. 2b). The selectivity of **1** toward a range of physiological and environmentally important metal ions was investigated by preparing solutions containing 10 μM of probe and 50 μM metal ion. The results (Fig. 2a) show that a significant quench (90%) of the original emission intensity was observed for Fe(III) with a much smaller quench (50%) observed for Cu(II) while the remaining ions had a negligible effect. The quenching effect of Fe(III) and to a lesser extent Cu(II) is most likely due to electron transfer from these redox active metal ions to the excited state of **1** leading to non-radiative decay of the excited state [16, 17]. Upon the continuous addition of Fe(III) to a solution of **1**, a concentration dependent reduction in the intensity at 507 nm was observed, with good linearity established in the 0.05 to 0.20 mM range. Based on a 1:1 binding stoichiometry the binding constant (logβ) was calculated using the equation $\log(F_{MAX} - F)/(F/F_{MIN}) = \log[\text{anion}]$—logβ the binding constant (logβ) and found to be 4.01 [18].

To determine the effect of solution pH on the fluorescence response of **1** we conducted a pH titration (Fig. 3). From pH 2–11 the fluorescence intensity remained relatively unchanged meaning **1** is effective over a broad pH range. In addition, we performed a competitive titration where equimolar amounts of Cu(II) and Fe(III) were added to a solution of **1** (Fig. 4). These results showed that it was possible to measure Fe(III) concentration in the presence of Cu(II) up to a concentration of 0.14 M.

Conclusions

In summary, we have prepared a new Schiff base probe capable of detecting Fe(III) by changes in its fluorescence emission in semi aqueous solution. In addition, this probe also enables the naked eye detection of Fe (III) from a range of other biologically and physiologically important cations. The probe showed good selectivity over other tested ions, is stable over a broad pH range and can detect Fe(III) in competition with Cu (II) in the 0.05–0.14 mM range.

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