A SIMPLE AND EASY-TO-PREPARE IMIDAZOLE-BASED PROBE FOR THE SELECTIVE CHROMO-FLUROGENIC Cu(II) DETECTIOIN IN AQUEOUS ENVIRONMENTS

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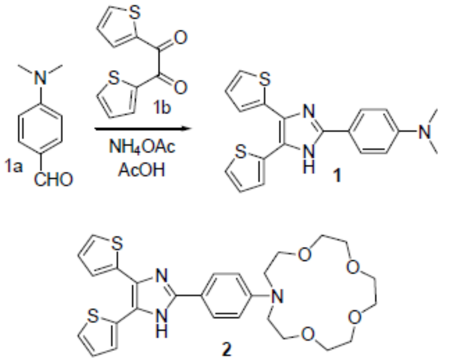
ABSTRACT: A new simple and easy-to-prepare Imidazole-based probe **1** was synthesized and used to detect Cu(II) cation in aqueous environments. At this respect, addition of increasing amounts of Cu(II) to water (pH 7.4)-acetonitrile 90:10 v/v solutions induced the appearance of a red-shifted absorption together with a marked color change from colorless to deep blue. Also probe **1** is fluorescent and a marked emission quenching in the presence of Cu(II) cation was observed. The optical response is quite selective because, of all the cations tested, only Cu(II) induced marked changes. Besides limits of detection for Cu(II) of 0.7 and 3.2 µM using UV-visible and fluorescence measurements were determined.

INTRODUCTION

Transition metal cations are involved in several vital process and are also used as diagnostic tools in medical, physiological and environmental fields [1-3]. Thus, the development of techniques for monitoring transition metal cations is an active area of research. Among the various transition metal cations, Cu(II) is the third most abundant essential element in the human body due to its vital role in many physiological processes [4-9]. In addition, it was found that Cu(II) cation stimulates the proliferation of endothelial cells and is necessary for the secretion of several angiogenic factors by tumour cells [10,11]. Apart from its biological and environmental importance, copper is widely used in the metallurgical, pharmaceutical and agrochemical industries [12]. As a result of the extensive applications of Cu(II) in life science and industry, it has become one of the first hazard environmental pollutants [13]. Although the important role played by Cu(II), abnormal levels cause serious health problems on human beings due to its ability to displace other vital metal ions in some enzyme-catalysed reactions [14]. In addition, high concentrations of Cu(II) in cells was documented to cause toxicity and different neurodegenerative diseases such as Menkes, Wilson's and Alzheimer [15]. Therefore, simple and rapid sensing tools to monitor Cu(II) levels in biological and environmental mediums is of great importance.

In the past years, electrochemical methods, spectrometry and chromatography have been used to detect Cu(II) cation. However, these methods are usually limited by high costs, are time consuming and are not prepared for in situ and at site analysis. As an alternative to these classical methods, the use of chemical optical probes has attracted great attention in the last years and several Cu(II) sensors were reported. Some of these probes are able to detect Cu(II) cation both in solution (by colour and/or emission changes) and even in living cells (by using confocal microscopy) [16-20]. In spite of these interesting features, most of these probes operate only in organic solvents and often presented poor selectivity [21,22]. Thus, the preparation of selective probes that can detect Cu(II) cation in water or water/organic solvents is still of a matter of concern.

Bearing in mind our experience in the development of molecular probes for detection of anions and cations of biological and environmental significance [23-27], we report herein the synthesis and sensing features of a new imidazole-based easy-to-prepare chromo-fluorogenic probe **1** able to detect Cu(II) cation in water (pH 7.4)-acetonitrile 90:10 v/v mixtures. The synthesis of probe 1 was achieved in one step using a condensation reaction between 4-(dimethylamino) benzaldehyde (1a) and 1,2-di(thiophen-2-yl)ethane-1,2-dione (1b) in acidic medium (see Scheme 1n for details). ^{1}H NMR spectrum of probe **1** in DMSO-d_6 (see experimental section and Supporting Information) showed a singlet centred at 2.96 ppm that could be ascribed to the *N*-dimethylamino protons.



Scheme 1. Synthesis of probe 1 and structure of closely related macrocycle-containing imidazole-derivative 2.

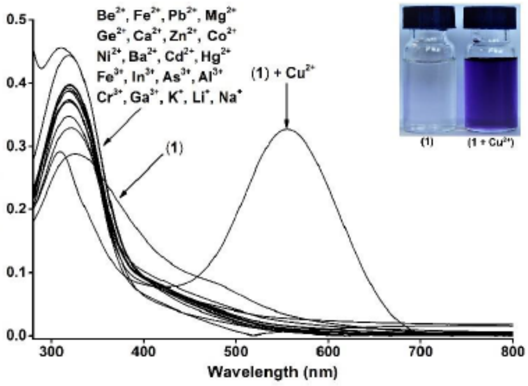


Figure 1. UV-visible spectra of probe 1 in water (pH 7.4)-acetonitrile 90:10 v/v (5.0 x 10^{-5} mol L^{-1}) alone and in the presence of 10 eq. of selected metal cations. The inset shows the change in colour of probe 1 in the presence of Cu(II) cation.

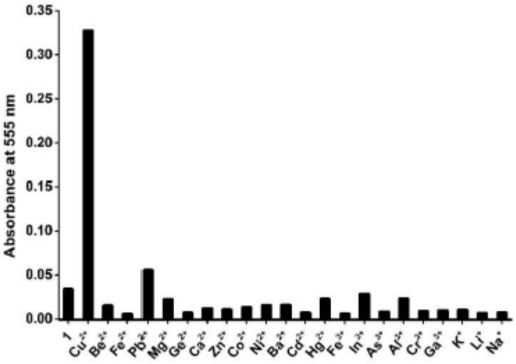


Figure 2. Absorbance at 555 nm of water (pH 7.4)-acetonitrile 90:10 v/v (5.0 x 10^{-5} mol L^{-1}) solutions of probe 1 alone and in the presence of selected metal cation (10 eq.).

The most remarkable signals in the aromatic zone are two well defined doublets at 6.77 and 7.84 ppm ascribed to the 1,4-disubstituted benzene ring. On the other hand the six thiophene protons appeared in the 7.00-7.70 ppm interval. Finally, the imidazole proton appeared as a broad singlet centred at 12.46 ppm.

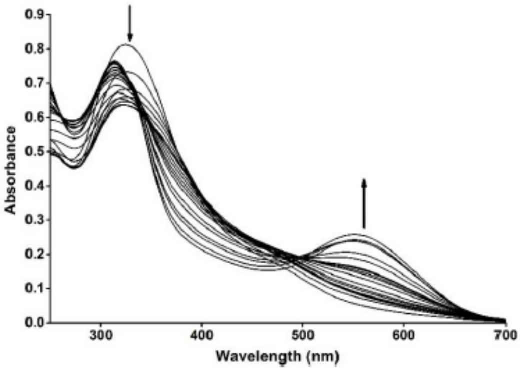


Figure 3. UV-visible titration profile of probe 1 in water (pH 7.4)-acetonitrile 90:10 v/v (5.0 x 10^{-5} mol L^{-1}) upon addition of increasing amouts of Cu(II) cation (from 0 to 10 eq.).

Probe **1** is not completely water soluble and, for this reason, the spectroscopic behaviour was studied in water (pH 7.4)-acetonitrile 90:10 v/v solutions. At this respect, water (pH 7.4)-acetonitrile 90:10 v/v solutions of probe **1** (5.0 x 10^{-5} mol L^{-1}) presented an absorption band centred at ca. 320 nm (see Figure **1**). Next, UV-visible changes in probe **1** upon addition of 10 eq. of Cu(II), Pb(II), Mg(II), Ge(II), Ca(II), Zn(II), Co(II), Ni(II), Ba(II), Cd(II), Hg(II), Fe(III), In(III), As(III), Al(III), Cr(III), Ga(III), K(I), Li(I) and Na(I) cations was studied. As could be seen in Figure 1, of all the cations tested, only Cu(II) was able to induce the appearance of a new absorption band centred at 555 nm. The marked changes in the UV-visible spectrum of probe **1** upon addition of 10 eq. of Cu(II) cation is reflected in a clear colour change from colourless to deep blue (see also Figure 1). Besides, Figure 2 showed the intensity of the band centred at 555 nm of aqueous solutions of probe **1** in the presence of selected cations. As could be seen, the response of probe **1** toward Cu(II) is highly selective because the other cations tested induced negligible changes in the 550 nm band.

Having assessed the highly selective response of probe **1** toward Cu(II) cation we studied, in the next step, the changes in the UV-visible spectra upon addition of increasing amounts of Cu(II). As could be seen in Figure **3** addition of increasing quantities of Cu(II) cation to water (pH 7.4)-acetonitrile 90:10 v/v solutions of probe 1 (5.0 x 10^{-5} mol L^{-1}) induced the progressive decrease of the absorption centred at 320 nm together with the growth of the visible band at 555 nm. Besides, during the course of the titration, isosbestic pints appeared at ca. 340 and 500 nm. From the obtained titration profile a limit of detection of 0.7 μM was determined (see Supporting Information) which is almost 100 times lower than the limit prescribed by the World Health Organization (WHO) guideline for drinking water (30 mM). [28, 29]

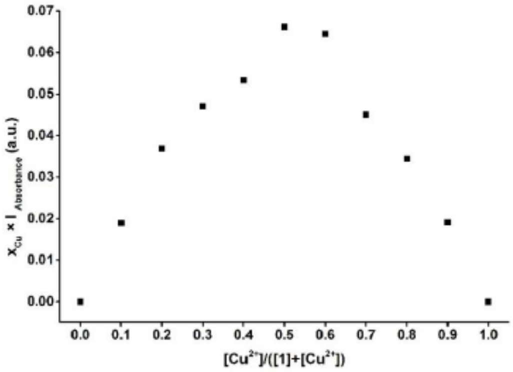


Figure 4. Job's plot of probe 1 and Cu(II) in water (pH 7.4)-acetonitrile 90:10 v/v. Total concentration of 1 and Cu(II) of 2.0 x 10^{-5} mol L^{-1}.

In addition, probe **1** present high competitive features in water (pH 7.4)-acetonitrile 90:10 v/v more than various recent peer reviewed articles. Zheng and coworkers reported a detection limit of 2.13 mM for Cu(II) ion in DMSO/H_2O (1 : 1 v/v) while Xu and coworkers reported a detection limit of 1.63 mM for Cu(II) ion in EtOH/H_2O(4 : 1 v/v, 10 mM HEPES, pH 7.4).[30,31]

In order to understand the mode of coordination between probe **1** and Cu(II) cation Job's plot were measured. As could be seen in Figure 4, probe **1** clearly forms a 1:1 stoichiometry complex with Cu(II) cation. From the UV-visible titration showed in Figure 3 a logarithm of the stability constant for the formation of 1:Cu(II) complex of 3.50 ± 0.15 was determined.

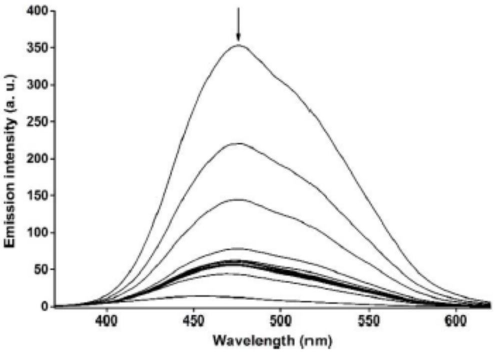


Figure 5. Fluorescence titration profile of probe 1 in water (pH 7.4)-acetonitrile 90:10 v/v (5.0 x 10^{-5} mol L^{-1}) upon addition of increasing amounts of Cu(II) cation (from 0 to 10 eq.).

The obtained emission quenching is remarkable; taking into account, the results obtained with the parent probe 2 (see Scheme 1). At this respect, ethanol solutions of macrocycle-containing probe **2** presented a weak emission band that was markedly increased upon addition of Cu(II) cation. The marked emission enhancement observed with **2** could be ascribed to an increase in the rigidity of the probe upon formation of 2:1 metal-probe stoichiometry complexes (one Cu(II) cation coordinated with the macrocycle and the other with the nitrogen atoms of the imidazole heterocycle with a logarithm of the stability constant of 11.58 ± 0.01). In our case, the fluorescence experiments are carried out in a more competitive media (water-acetonitrile 90:10 v/v) and probe **1** lacks the macrocycle binding domain presented in **2**. Taking into account the red shift observed in the UV-visible titration profile of probe **1** with Cu(II), and also the formation of 1:1 stoichiometry complex assessed from the Job's plot, we proposed that this cation coordinates with one of the nitrogen atoms of the imidazole heterocycle. The observed quenching of the emission intensity of **1** upon Cu(II) binding is a contribution of an electron or energy transfer process between the probe and the cation. Also a reabsorption process (due to the partial overlap between the new absorption band of the formed complex and the emission of the probe alone) could not be discarded.

In summary, we report herein an easy-to-prepare imidazole-based chromo-fluorogenic probe **1** for the selective and sensitive optical detection of Cu(II) cation. Probe **1** was able to selectively detect Cu(II) cation in a highly competitive media (water-acetonitrile 90:10 v/v) by a marked colour change from colourless to deep blue. Besides, a remarkable quenching of the probe emission in the presence of Cu(II) cation was observed.

ACKNOWLEDGMENT

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