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Selective ratiometric detection of Hg²⁺ in pure water using a phenoxazinium-based probe

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ABSTRACT

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The mercury(II) ion is considered to be one of the most toxic metal cations in the environment and is known to seriously affect human health.¹ Sensitive organic ligands for the detection of Hg²⁺, which can provide immediate optical feedback and which do not require sophisticated instrumentation and sample preparations, are highly valued. Many detection methods for Hg²⁺ based on organic small-molecules have been examined.² Current research shows that: (1) Ratiometric monitoring³ is a superior design for a probe because it can increase the selectivity and sensitivity of the measurement and eliminate most or all of the interfering factors by self-calibrating the two emission or absorption bands.⁴ (2) It is very important that the probe has absorption or emission bands in the longer wavelengths.^{2b,3g,5} Light in the near-infrared (NIR) region (650–900 nm)⁶ causes minimal photo damage⁷ and can avoid the influence of autofluorescence.⁸ (3) A fully watersoluble probe is an important goal, as it facilitates a probe's application in environmental or biological fields of study.⁶

However, few aqueous probes^{3a,b,10} have been reported when compared to the large number of organic^{2a} or water-compatibile^{2b-d} probes. In light of these precedents, we committed ourselves to the development of a new water-soluble ratiometric sensor with long-range excitation and emission as well as prospects for environmental or biological utility.

The symmetric and unsymmetrical 3,7-bis(dialkylamino)-phenoxazin-5-ium derivatives, which contain a phenoxazinium cation with absorbs in the 640-650 nm region and have corresponding

A ratiometric, near-infrared, and fully water-soluble probe, a phenoxazinium-based chemosensor bearing an anilino thiaazacrown, was successfully synthesized and characterized. The use of this probe for the selective ratiometric detection of Hg^{2+} in pure water is reported. The probe shows good selectivity for Hg^{2+} , and a large blue shift (75 nm) of the complex's absorption maximum was observed.

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Scheme 1. Synthesis of phenoxazinium 1a.

emission bands between 650 and 670 nm,¹¹ have comparatively low toxicity according to previous in vitro and in vivo assay results.^{12a,b} We assume that 3,7-bis(dialkylamino)phenoxazin-5ium derivatives that are appended with a 1,4-dioxa-7,13-dithia-10-azacyclopentadecane unit (e.g., 1a) coordinate with Hg^{2+} through the anilino thiaazacrown.^{2b,3a,d} The absorption and emission properties are modulated by the extended $p-\pi$ conjugation between the nitrogen atom and phenoxazinium.¹¹



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Figure 1. (a) Changes in the UV-vis spectra of $\mathbf{1a}$ (50 × 10⁻⁶ M in water) upon titration with Hg(AcO)₂ from 50 × 10⁻⁶ M to 300 × 10⁻⁶ M. Inset: color change of $\mathbf{1a}$ in the visible region. (i) $\mathbf{1a}$ solution in water; (ii) (i) + 6 equiv Hg²⁺. (b) Changes in the UV-vis spectra of $\mathbf{1a}$ (40 × 10⁻⁶ M in water) upon addition of 5 equiv mercury ion and subsequently an excess of EDTA. Inset: color change of $\mathbf{1a}$ in the visible region. (i) $\mathbf{1a}$ solution in water; (ii) (i) + 5 equiv.Hg²⁺; (iii) (ii) + excess of EDTA. (c) Job plot analysis of $\mathbf{1a}$ and Hg²⁺ in water, the total molar concentration of $\mathbf{1a}$ and Hg²⁺ is 60 × 10⁻⁶ M.

3-(Diethylamino)-7-(1,4-dioxa-7,13-dithia-10-azacyclo pentadecan-10-yl) phenoxazin-5-ium chloride (**1a**) was obtained in 30% yield by reacting 1,4-dioxa-7,13-dithia-10-(3-methoxy-4nitrosophenyl)-10-azacyclopentadecane (**4**) with 3-diethyl-aminophenol (Scheme 1). Compound **1a** exhibits good solubility in water and polar solvents, such as methanol and chloroform.

Figure 1a shows the changes in the absorption spectrum of **1a** as a function of Hg^{2+} concentration in water at room temperature. The UV–vis spectrum of **1a** in water is characterized by a very intense band centered at 653 nm ($\varepsilon = 76000 \text{ M}^{-1} \text{ cm}^{-1}$) that is responsible for the light blue color of the solution. During photometric titrations of **1a** with Hg^{2+} , a blue shift of the long-wavelength absorption maximum from 653 nm to 578 nm ($\varepsilon = 34000 \text{ M}^{-1} \text{ cm}^{-1}$) (with one isosbestic point at 590 nm) was observed, which may be the result of the complexation of Hg^{2+} with the crown ether unit. Such a large blue-shift (75 nm) makes the color of the solution change from light blue to dark blue (Fig. 1a). The subsequent addition of an excess of ethylenediamine tetra-acetic acid (EDTA) resulted in recovery of the original color (Fig. 1b).

According to the linear Benesi–Hildebrand expression^{2a}, the measured absorbance [1/(A-A₀)] at 650 nm varied as a function of 1/[Hg²⁺] in a linear relationship indicating the 1:1 stoichiometry between the Hg²⁺ ion and **1a** (Fig. S4b, Supplementary data). On the basis of 1:1 stoichiometry and UV–vis titration data in Figure 1a, the association constant of **1a** with Hg²⁺ ion in water was found to be 4.5 × 10³ M⁻¹ (Fig. S4b, Supplementary data). Job plot analysis^{2e} of the UV–vis titrations carried out in water revealed a maximum at a 50% mole fraction, in accord with the proposed 1:1 binding stoichiometry(Fig. 1c).

The absorption spectra of $1a Hg^{2+}$ clearly indicate that the absorption band at 653 nm has shifted and developed a new band at 578 nm. The blue-shifted absorption indicates that the complex formed between 1a and Hg^{2+} alters the properties of the amino functionality, significantly suppressing its ability to donate electron density to the phenoxazinium chromophore.

The complexation of Hg^{2+} with compound **1a** was demonstrated by proton nuclear magnetic resonance spectra (¹HNMR) spectroscopy (Fig. 2). Thus, upon addition of Hg^{2+} , the aliphatic protons of the methylene units next to the sulfur atoms, H-b and H-c, experience large downfield shifts of 0.55 ppm and 0.46 ppm, respectively, whereas the methylene protons adjacent to the oxygen atoms, H-d and H-e, show slight downfield shifts (0.09 ppm). The chemical shifts of the aromatic protons concurrently move to lower field (0.09–0.28 ppm), indicating the reduced donor strength of the amino substituent as a result of the complexation of **1a** with Hg^{2+} . The peaks corresponding to the protons at C-8 and C-6, which are located at the β -position of the amino group, are particularly useful probes to detect the influence of the Hg^{2+} complexation. Addition of Hg^{2+} to **1a** induces a large downfield shift of the 8-H and 6-H signals, which also clearly indicates a decrease in the electron donating ability of the amino group.

Control experiments were performed to evaluate the selectivity of probe **1a** toward Hg²⁺. The influence of the addition of Hg²⁺, Na⁺, Mg²⁺, Ca²⁺, K⁺, Cd²⁺, Zn²⁺, Ni²⁺, Cr³⁺, Cu²⁺, Pb²⁺, Mn²⁺, Fe³⁺, or Ag⁺ on the absorption properties of **1a** was studied (Fig. 3a). The concentration of the metal ions was 0.1×10^{-3} M, except for K⁺, Na⁺, Mg²⁺, and Ca²⁺ (100×10⁻³ M) because the latter would likely be present in higher concentrations in real samples. It should be noted that the addition of Hg²⁺ leads to a significant change of the absorption of **1a**, a blue-shift in the absorption maximum of 75 nm was observed, and the solution color changed from light blue to dark blue (Fig. 1a). A slight decrease in the absorption intensity was detected upon addition of Ag⁺ (Fig. 3a); and the competition experiments show that it has no significant influence when the samples contained different metal cations (Fig. S2a, Supplementary data).

The absorption intensity ratio I_{530}/I_{653} (Fig. 3b) of the UV–vis spectrum shows that the shift of the absorption maximum of **1a** upon complexation with Hg²⁺ allows the analysis of the UV–vis titration by a ratiometric plot, that is, a plot of I_{530}/I_{653} versus Hg²⁺ concentration. In contrast, there is almost no change in the I_{530}/I_{653} ratio (0.18) upon addition of Cd²⁺, Zn²⁺, Ni²⁺, Cr³⁺, Cu²⁺, Pb²⁺, Co²⁺, Fe³⁺, Na⁺, Mg²⁺, or Ca²⁺. However, the addition of Ag⁺ induced a slight increase of the I_{530}/I_{653} value (0.39). At the employed concentration of the probe (50 × 10⁻⁶ M), the limit of detection for Hg²⁺ is 10⁻⁶ M, and the linear range of the detection is 20–120 × 10⁻⁶ M (Fig. S1a and S1b Supplementary data).

3,7-Di(piperidinyl)phenoxazin-5-ium chloride(**1b**, Scheme 2) was used to practice the same procedure, and there was not any difference in the absorption spectra(Fig. S3a, Supplementary data). This indicated that the absorption is changed not by possible combination¹³ between the phenoxazinium skeleton and Hg²⁺ but by the chelation between the anilino thiaazacrown and Hg²⁺. Regarding possible applications of ligand **1a**, these conditions permit the detection and quantification of Hg²⁺ in industrial waste water. Note that fluorometric detection of Hg²⁺ is also possible with

Note that fluorometric detection of Hg^{2+} is also possible with compound **1a**. The addition of Hg^{2+} causes a decrease and a blue-shift in the fluorescence of compound **1a**. As there is slight decrease of the fluorescence intensity of **1b** and Hg^{2+} (Fig. S3b, Supplementary data), we assume that the emission is mainly changed by the combination between the anilino thiaazacrown with Hg^{2+} , and it is also slightly influenced by the chelation between the phenoxazinium skeleton and Hg^{2+} .

To determine the amount of Hg^{2+} required to induce fluorescence quenching of **1a**, titration experiments were performed (Fig. 4); when 6 equiv of Hg^{2+} was added, the emission of **1a** at



Figure 2. Top: ¹H NMR spectra of free **1a** in CD₃OD. Bottom: ¹H NMR spectra of **1a** in the presence of Hg²⁺ (2 equiv) in a mixed solvent system of D₂O and CD₃OD (D₂O: CD₃OD = 1:5).



Figure 3. (a) Changes in the UV-vis spectra of **1a** (50×10^{-6} M in water) upon addition of different metal cations (0.1×10^{-3} M: Hg²⁺, Cd²⁺, Zn²⁺, Ni²⁺, Cr³⁺, Cu²⁺, Pb²⁺, Fe³⁺, Ag⁺, Mn²⁺; 100×10^{-3} M: K⁺, Na⁺, Mg²⁺, Ca²⁺). (b) Relative absorption intensities of the ligand **1a** (c = 50×10^{-6} M), given as the ratio I_{530}/I_{653} upon addition of different metal cations.



Scheme 2. Structure of phenoxazinium 1b.



Figure 4. Emission spectra of compound **1a** in the presence of increasing Hg²⁺ concentration (from 50×10^{-6} M to 300×10^{-6} M) in water. The excitation wavelength was 590 nm with a 5 nm slit width. The concentration of **1a** was 50×10^{-6} M.

695 nm was quenched and blue-shifted. The detection limit of the sensor at this concentration for Hg²⁺ is about 50×10^{-6} M (Fig. S1c, Supplementary data). The control experiments and competition experiments were also performed to evaluate the selectivity of probe **1a** toward Hg²⁺ upon fluorescence spectra. The experiments indicate the probe **1a** has high selectivity toward Hg²⁺ using fluorescence spectra (Fig. S2b and S2c Supplementary data).

The emission of Hg^{2+} complexed **1a** is quenched at low pH (Fig. S4a, Supplementary data), presumably owing to protonation of the complex in the exited state because neither the absorption of **1a** in the absence or the presence of Hg^{2+} (0.1×10^{-3} M), nor the emission of uncomplexed **1a** change with decreasing pH.^{3a} These results demonstrate the pH dependence of the fluorometric performance of 3,7-bis(dialkylamino) phenoxazin-5-ium conjugates. Therefore, if the pH of the medium is known, compound **1a** is still an opportune Hg^{2+} sensitive probe as demonstrated by calibration curves determined at given pH values.^{3a}

In conclusion, we present an example of the ion probe's selection for the suitable chromophore or fluorophore from the existing or potential drug molecules. A phenoxazinium-based chemosensor bearing an anilino thiaazacrown (**1a**) as a promising analytical tool for UV-vis and fluorometric mercury detection with long-range excitation and emission. Specifically, this probe is one of the few fluorescent probes that allows for the selective ratiometric detection of mercury ions in pure water with no organic co-solvent required. A large blue-shift (75 nm) of the absorption maximum of **1a** was observed upon titration with Hg^{2+} , and the solution's color changed simultaneously from light blue to dark blue. Importantly, the selectivity of the probe for Hg^{2+} over other metal ions is extremely high.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.03.013.

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