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Novel hemicyanine dye as colorimetric and fluorometric dual-modal chemosensor for mercury in water†

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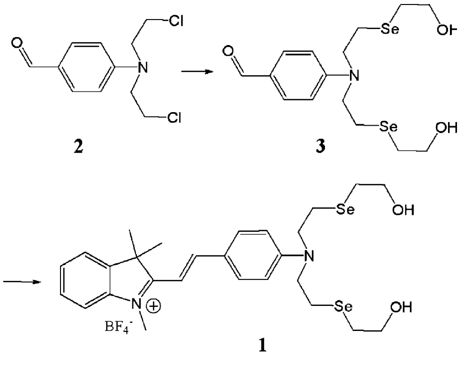
A novel water soluble Hg^{2+}-selective chemosensor 1 with hemicyanine as fluorescent reporting group and NO_2Se_2 chelating unit as ion binding site was reported. Chemosensor 1 shows a specific Hg^{2+} selectivity and discrimination between Hg^{2+} and chemically similar ions in conjunction with a visible colorimetric change from red to colorless, potentially leading to both "naked-eye" and fluorometric detection of Hg^{2+} cations.

The development of highly selective chemosensors for metal ions is particularly important, since some metal ions can have extremely harmful effects on humans and the environment.[1] Mercury is a particular concern worldwide because of its high toxicity. Mercury species are considered as prevalent toxic pollutants in the environment because both elemental and ionic mercury can be converted by bacteria in the environment to methylmercury, which subsequently bioaccumulates through the food chain.[2] When absorbed in the human body, mercury causes a wide variety of diseases such as prenatal brain damage, serious cognitive and motion disorders[2b,3] through damage to the central nervous system, DNA, mitosis, and the endocrine system.[4] Thus, concerns over toxic damage from mercury provide the motivation to explore the selective monitoring of Hg^{2+} in biological and environmental samples. Since chromo- or fluoroionophores are proven to be highly effective for the determination in terms of ease of handling and simplicity of equipment, much effort has been paid to the development of optical chemosensors that selectively respond to the mercuric ion.[5] To date, many chemosensors for mercury ion based on photoinduced electron transfer (PET), intramolecular charge transfer (ICT), and fluorescence resonance energy transfer (FRET) signaling mechanisms have been developed.[5] Dosimeters[6] and allosteric interaction sensors[7] have also been reported. Significantly, the molecular design of multimodal chemosensors for mercury is still a challenging but extremely attractive method because they have important features that permit multimodal signal detection when receptors interact with analytes; a multimodal signal system can minimize the measurement errors because of factors such as phototransformation, receptor concentrations, and environmental effects. In particular, some of them can be easily read with the naked eye. Consequently, the development of multimodal and naked eye chemosensors for Hg^{2+} has the important feature that they can be used to evaluate the analytes rapidly. In particular, most of the reported colorimetric sensors for Hg^{2+} are used in mixed solvents (water/organic solvent);[6g,8] only a few of them are used in neat aqueous solution.[5j,9]

In this regard, we are interested in hemicyanine-based dyes, known for their excellent spectroscopic properties of large molar extinction coefficient ($\varepsilon \approx 10^4$ M^{-1} cm^{-1} in the visible region) and good fluorescence quantum yield, and which have found applications in many fields, such as the study of complex biological systems as molecular probes, photosensitizers in dye-sensitized solar cells *etc*, but few as chemosensors.[10] Taking into account the possibility of using positively charged hemicyanine dyes not only for colorimetric but also for fluorometric mercury sensing, increasing the water solubility and sensitivity by using fluorescence emission spectroscopic methods, we report our successful development of a novel hemicyanine-based water soluble colorimetric and fluorometric chemosensor **1** selective for Hg^{2+} based on the mechanism of internal charge transfer (ICT).

We report herein a new hemicyanine-based water soluble chemosensor **1** (Scheme 1), which displayed high Hg^{2+} selectivity both in water and in ethanol–water solution. The chemosensor **1** was synthesized from **2** in two steps. As shown in Scheme 1, aldehyde **3** was prepared by the reaction of **2** with sodium salt



Scheme 1 Structure and the synthesis of the chemosensor **1**.

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of 2-selenolethanol prepared *in situ* by the reaction of 1,2-(bis-2-hydroxylethyl)diselenide with $NaBH_4$ in THF–ethanol mixture. Then, **1** was facilely obtained by the condensation of 1,2,3,3′-tetramethylindolium iodide with **3** in 83% yield. The anion exchange was achieved by refluxing the ethanol solutions **1** with excess KBF_4. The structure of **1** was confirmed by MS, NMR and elemental analysis. The synthesized chemosensor **1** composed of an ionophore for selective recognition of metal ions is constituted of an NSe_2O_2 chelating unit as cation binding site as well as a hemicyanine fluorophore unit which is responsible for signal transduction during spectroscopic studies.

Chemosensor **1** is soluble in H_2O or in a mixed solvent of H_2O–EtOH. The UV/Vis spectra of **1** both in H_2O and in aqueous ethanol show the characteristic intense charge-transfer band occurring in the long-wavelength region between 425 and 600 nm, which peaked at 529 nm in water ($\varepsilon = 7.1 \times 10^4$ M^{-1} cm^{-1}) and 542 nm in EtOH–H_2O (1 : 1, v/v) ($\varepsilon = 8.3 \times 10^4$ M^{-1} cm^{-1}) (Fig. 1a and 1b). It is interesting to note that the addition of 10 equiv of Hg^{2+} into the red solutions of **1** rapidly generated a colorless solution, while other ions, such as K^+, Na^+, NH_4^+, Al^{3+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Ag^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+} gave no color change in either H_2O or in aqueous ethanol, which means that the two selenium atoms within **1** bound more efficiently to with Hg^{2+}. This interesting feature revealed that **1** can serve as a selective "naked-eye" chemosensor for Hg^{2+} (Fig. 2).

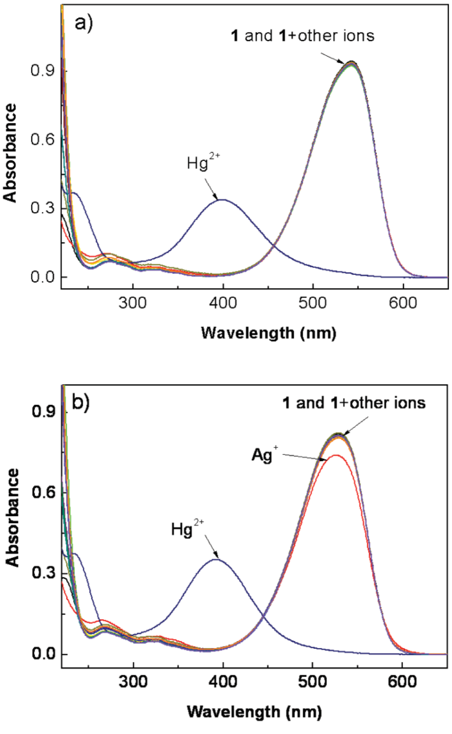

Fig. 1 UV-vis responses of chemosensor **1** (11.5 μM) upon the addition of the nitrate salts (10.0 equiv) of Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Pb^{2+}, Hg^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Fe^{3+} and Ag^+ (a) in ethanol–H_2O (1 : 1, v/v) and (b) in H_2O.

The metal ion binding properties and selectivity of chemosensor **1** were firstly investigated by absorbance changes both in H_2O and in aqueous ethanol (ethanol–water, 1 : 1, v/v) solutions with use of metal nitrate salts. Fig. 1a displays the absorbance changes of **1** in the presence and absence of metal ions in EtOH–H_2O (1 : 1, v/v). As can be seen from Fig. 1a, the response of **1** to Hg^{2+} leads to a variation in absorption. When 10 equiv of Hg^{2+} was added to the solution of **1** (11.5 μM), the absorption peak at 542 nm diminished drastically, while a new absorption band appeared at 400 nm ($\varepsilon = 2.0 \times 10^4$ M^{-1} cm^{-1}). There was a large decrease (54-fold) of absorbance at $\lambda_{max} = 542$ nm and a mild enhancement (42-fold) of absorbance at $\lambda_{max} = 400$ nm upon the addition of 10 equiv of Hg^{2+}. Fig. 1b displays the absorbance changes of **1** in the presence of metal ions in water. When 10 equiv of Hg^{2+} was added to the solution of **1** (11.5 μM), the absorption peak at 529 nm diminished drastically, while a new absorption band appeared at 384 nm ($\varepsilon = 1.7 \times 10^4$ M^{-1} cm^{-1}). There was a large decrease (74-fold) of absorbance at $\lambda_{max} = 529$ nm and a mild enhancement (27-fold) of absorbance at $\lambda_{max} = 384$ nm upon the addition of 10 equiv of Hg^{2+} (Fig. 1b). As shown in Fig. 1b, under identical conditions to Hg^{2+} ion, small increases in absorbance at $\lambda_{max} = 529$ nm were observed in the UV/vis spectra of **1** upon addition of 10 equiv of K^+, Na^+, NH_4^+, Al^{3+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+} but Ag^+ decreases the absorption to some extent in H_2O. From these results, it can be considered that the decrease of absorption intensity over 500 nm and the increase of absorption intensity around 400 nm caused by Hg^{2+} addition are due to the change in the photophysical properties of the chromophore itself, and arise *via* the ICT mechanism.

Fig. 2 Photograph of 10 μM **1** in the presence of different nitrate salts: (10.0 equiv) of Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Pb^{2+}, Hg^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Fe^{3+} and Ag^+ in H_2O.

The absorbance changes of Hg^{2+} titration of **1** were investigated both in H_2O and in aqueous ethanol (ethanol–water, 1 : 1, v/v) solutions. As shown in Fig. 3, upon addition of Hg^{2+} to the solution of **1**, the absorbance at 542 nm in aqueous ethanol and 529 nm in water decreased sharply, while that at 412 nm in aqueous ethanol and 404 nm in water increased significantly, which induced a color change from red to colorless. The isosbestic points at 471 nm (in EtOH–H_2O, 1 : 1, v/v) and 457 nm (in H_2O) were observed, indicating the formation of a new complex between **1** and Hg^{2+}. A linear dependence of the ratio of absorbance as a function of Hg^{2+} concentration both in water and in aqueous ethanol was observed.

Next, the nitrate salts of K^+, Na^+, NH_4^+, Al^{3+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Ag^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+} and Hg^{2+} ions were used to evaluate the metal ion binding properties and the selectivity of chemosensor **1** (1 μM) by means of fluorescence spectra in EtOH–H_2O (1 : 1, v/v) and in H_2O, respectively. Among the metal ions examined, **1** showed a selective fluorescence decrease only with Hg^{2+} both in EtOH–H_2O (1 : 1, v/v) and in H_2O, indicating that **1** displayed a high Hg^{2+} selectivity (Fig. 4 and Fig. S1, ESI†). The fluorescence spectra were obtained by excitation of the hemicyanine fluorophore at 540 nm.

To achieve a reliable binding constant (K) *via* fluorescence titration,[11] the titrations were conducted in a very dilute solution (1.0×10^{-6} M). From the fluorescence titration experiments (Fig. 5), clear "on–off" fluorescence changes of **1** to Hg^{2+} were observed.

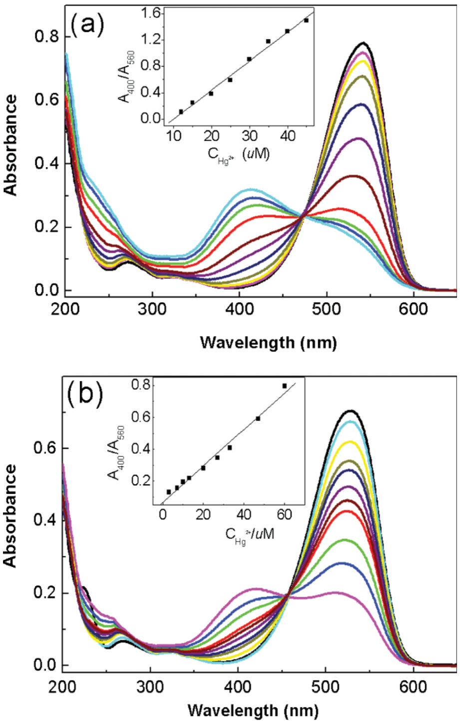

Fig. 3 Absorbance spectra of **1** (10 µM) (a) in EtOH–H_2O (1 : 1, v/v) and (b) in H_2O in the presence of different amounts of Hg^{2+}. Inset: the ratio of absorbance at 560 nm and absorbance at 400 nm as a function of Hg^{2+} concentration.

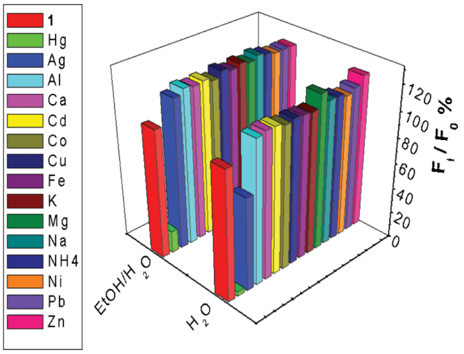

Fig. 4 Fluorescence spectra of **1** (1 µM) in ethanol–water (1 : 1, v/v) and in H_2O with 10 equiv of K^+, Na^+, NH_4^+, Al^{3+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Ag^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+} and Hg^{2+}. For the entire test, excitation and emission were performed at 540 and 596 nm in ethanol–water (1 : 1, v/v) and at 520 and 591 nm in water.

When Hg^{2+} was added to the solution, a significant decrease of the fluorescence intensity of 596 nm (in aqueous ethanol)/587 nm (in water) and with a blue shift to 575 nm was observed. The quenching is found to follow a Benesi–Hildebrand equation and may be attributed to the nitrogen lone-pair electrons induced by the complexation of Hg^{2+}. A linear dependence of the ratio of fluorescence intensity [**1**][Hg^{2+}]/($F_0 - F_i$) at 594 nm (in aqueous ethanol)/590 nm (in water) as a function of Hg^{2+} concentration and the data from Job's plots from emission spectra (Fig. S2, ESI†) assumed a 1 : 1 stoichiometry for the **1**–Hg^{2+} complex. The binding constant was found to be 8.81×10^4 M^{-1} in aqueous ethanol and 4.30×10^5 M^{-1} in water, respectively, indicating that the binding ability of **1** in water is much stronger than that in aqueous ethanol. From the changes in Hg^{2+}-dependent fluorescence intensity (Fig. S3, ESI), the detection limit was estimated to be 5.0×10^{-8} M,[12a] indicating that the limit of detection of **1** to Hg^{2+} met the discharge limit for industrial waste water according to the China SA standard,[12b] or the US EPA standard.[12c]

Ion interference experiments were carried out both in H_2O and in aqueous ethanol (ethanol–water, 1 : 1, v/v) solvent systems for evaluation of the sensing ability of **1** toward Hg^{2+} in the presence of 50 equiv of a series of background metal ions, and the results are shown in Fig. 6. While Ag^+ had 5% background quenching

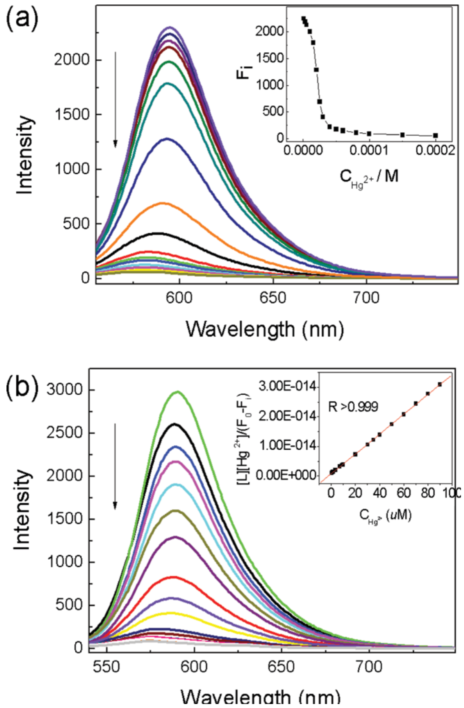

Fig. 5 Fluorescent titration spectra: (a) **1** (1 µM) in the presence of different concentrations of Hg^{2+} in ethanol–water (1 : 1, v/v), [Hg^{2+}] = 0, 1, 3, 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 150, 200 equiv. Inset: Fluorescence intensity F_i as a function of Hg^{2+} concentration. λ_{ex} = 540 nm; (b) **1** (1 µM) in the presence of different concentrations of Hg^{2+} in water, [Hg^{2+}] = 0, 0.4, 0.8, 1, 1.5, 2, 3.5, 7, 9, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90 equiv. Inset: the fluorescence at 590 nm of **1** (1 µM) as a function of the Hg^{2+} concentration. λ_{ex} = 530 nm.

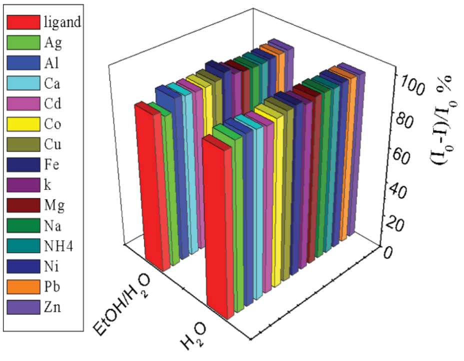

Fig. 6 Quench ratio (($I_0 - I$)/I_0) of fluorescence intensity of **1** (1 µM) in H_2O and in EtOH–H_2O (1 : 1, v/v) upon the addition of 10 equiv Hg^{2+} in the presence of 50 equiv background metal ions.

in aqueous ethanol, other metal ions did not cause any effect. No interference was observed in H_2O in the presence of 50 equiv of a series of metal ions. On the other hand, almost no color change is observed in the presence of metal ions other than Hg^{2+}. When 10 equiv of Hg^{2+} is added to these solutions, the red colour disappears immediately and the spectra are almost identical to that obtained in the presence of Hg^{2+} alone.

For practical applicability, the most suitable pH conditions of chemosensor **1** were evaluated by means of absorption spectra. The absorption intensities at different pH values of the apo and Hg^{2+}-bound forms of **1** in EtOH–H_2O (1 : 1, v/v) and in water solutions are shown in Fig. S4 and S5 (ESI†). From pH 2 to pH 10, absorption intensities are essentially insensitive to pH. Such photophysical properties of **1** in the presence and in the absence of Hg^{2+} suggest that this sensor may be very useful in practical applications.

In summary, we have reported here a simple yet highly selective colorimetric fluoroionophore (chemosensor **1**) for aqueous Hg^{2+} based on ICT in which the metal ionophore has been incorporated into the electron donor moiety of the fluorophore. The chemosensor shows a remarkably high ability to discriminate between Hg^{2+} and chemically similar ions in conjunction with a visible colorimetric change from red to colorless, leading to both "naked-eye" and fluorometric detection of Hg^{2+} cations.

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