

A Highly Sensitive and Selective Fluorescent Chemodosimeter for Hg^{2+} in Neutral Aqueous Solution

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Abstract A fluorescent assay of Hg^{2+} in neutral aqueous solution was developed using *N*-[*p*-(dimethylamino)benzamido]-*N'*-phenylthiourea (1). 1's fluorogenic chemodosimetric behaviors towards various metal ions were studied and a high sensitivity as well as selectivity was achieved for Hg^{2+} . It was because of a strongly fluorescent 1,3,4-oxadiazoles which was produced by the Hg^{2+} promoted desulfurization reaction. The spectra of ESI mass and IR provided evidences for this reaction. According to fluorescence titration, a good linear relationship ranging from 1.0×10^{-7} to 2.0×10^{-5} mol l^{-1} was obtained with the limit of detection as 3.1×10^{-8} mol l^{-1} .

Keywords *N*-[*p*-(dimethylamino)benzamido]-*N'*-phenylthiourea · Chemodosimeter · Fluorescence · Mercury ion

Introduction

Mercury is a very important element in environment and metabolizability because of its strong toxicity [1]. Mercuric

salts exist in many industrial materials such as electrical equipments, catalysts, paints, or as mining byproducts which produce high concentration of mercury pollutant. Accordingly, it is exigent to provide analytical methods for the sensitive and selective determination of mercury ion. Atomic absorption/fluorescence spectroscopy and inductively coupled plasma mass spectrometry were widely used in determining trace amount of mercury ion [2], which require expensive equipments and time consuming sample preparation. The development of alternative assays with easy operation and low cost has sparked great interest. Spectroscopic techniques employing fluorogenic sensors are especially appealing because fluorescence assay offers many advantages such as high sensitivity, selectivity, convenience, on-site or in situ analyses [3, 4]. It is well known that mercury ion might quench the emission of fluorescence reagents because of its open-shell, paramagnetic properties [5, 6]. Although there are some documents describing new assays for Hg^{2+} based on fluorescence quenching [7–10], methods showing fluorescence enhancement are favored because higher sensitivities can be achieved [11–27]. Among them, methods utilizing irreversible chemical reaction associated with host–guest recognition have been employed as chemodosimeters for the determination of Hg^{2+} concentrations [28–33]. In our studies, Hg^{2+} could promote desulfurization reaction of *N*-[*p*-(dimethylamino)benzamido]-*N'*-phenylthiourea (1) leading to the formation of a highly fluorescent 1,3,4-oxadiazoles in pH 7.4 aqueous solution. Consequent fluorescence increase was observed and fluorescence intensity was proportional to the Hg^{2+} 's concentration. We have previously reported the sensing of zinc ion by stable 1- Zn^{2+} complex formation [34].

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Experimental

Apparatus

All fluorescence measurements were carried out on a F-4500 spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp source and a 1.0 cm quartz cell, and the scan speed was 600 nm min^{-1} . Absorption spectra were recorded on a Shimadzu-2501 UV-VIS spectrophotometer (Japan) using a 1.0 cm quartz cell. All pH measurements were made with a PHS-3 digital pH-meter (Shanghai REX instrument factory, Shanghai, China) with a combined glass–calomel electrode. ^1H NMR spectrum was carried out in DMSO-d_6 on a Bruker Avance 500 MHz NMR spectrometer using TMS as the internal standard. ESI mass spectrum was recorded using a Waters ZQ4000/2695 LC-MS spectrometer. Elemental analyses data were obtained on a Vario EL III Elemental Analyzer.

Reagents

Compounds 1 and 2 were synthesized and characterized referring to the literature [34] and were characterized by IR, ^1H NMR and ESI mass data, which were consistent with proposed formulation. 1: ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 2.98 (6 H, s), 6.73 (2 H, d, $J=9$ Hz), 7.14 (1 H, t, $J=8$ Hz), 7.31 (2 H, t, $J=8$ Hz), 7.45 (2 H, d, $J=9$ Hz), 7.81 (2 H, d, $J=9$ Hz), 9.57 (s, NH), 9.71 (s, NH), 10.14 (s, NH); ESI mass: m/e calcd. for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{OS}$ $[\text{M}+\text{H}^+]$ 315.12,

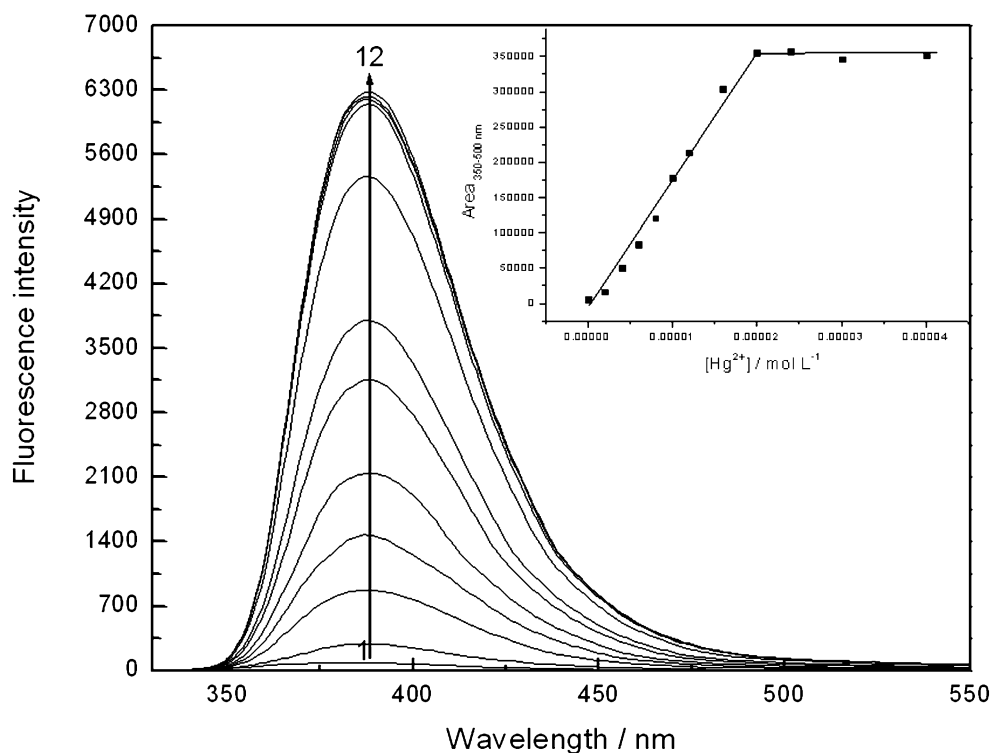
found $[\text{M}+\text{H}^+]$ 314.89; Anal. calcd for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{OS}$: C, 61.12; H, 5.77; N, 17.82%. Found: C, 61.03; H, 5.58; N, 17.77%. 2: ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 2.86 (3 H, s), 2.98 (6 H, s), 6.71 (2 H, d, $J=9$ Hz), 7.78 (2 H, d, $J=8.5$ Hz), 7.95 (1 H, s, NH), 9.18 (1 H, s, NH), 9.96 (1 H, s, NH); ESI mass: m/e calcd. for $\text{C}_{11}\text{H}_{16}\text{N}_4\text{OS}$ $[\text{M}+\text{H}^+]$ 253.10, found $[\text{M}+\text{H}^+]$ 252.89; Anal. calcd for $\text{C}_{11}\text{H}_{16}\text{N}_4\text{OS}$: C, 52.36; H, 6.39; N, 22.20%. Found: C, 51.87; H, 6.43; N, 22.38%. The stock solutions of compounds 1 and 2 were prepared in acetonitrile with a concentration of $1.0 \times 10^{-4} \text{ mol l}^{-1}$ for each. The concentration of mercury (II) chloride in water solution was $1.0 \times 10^{-4} \text{ mol l}^{-1}$.

Tris–HCl buffer of pH 7.4 contained 0.15 mol l^{-1} NaCl. Acetonitrile was of HPLC grade. All the chemicals used were of analytical grade and doubly distilled water was used throughout unless otherwise noted.

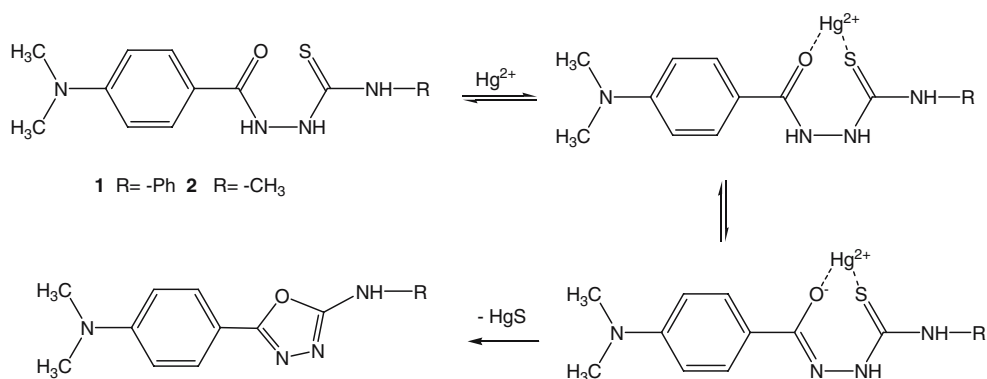
Procedure

To a 10 ml colorimetric tube, a certain amount of Hg^{2+} solution and 1 were added. Suitable amount of acetonitrile was added. The mixture was diluted to 10 ml with 0.01 mol l^{-1} Tris–HCl buffer. The final concentration of acetonitrile was 20% (v/v). The solution was fully mixed by shaking and heated in 60°C water bath for 5 min. The solution was cooled to room temperature. Fluorescence spectra were subsequently measured at excitation wavelength of 320 nm and the band-slit of both excitation and emission was set as 5.0 nm. The mass spectrum was

Fig. 1 The fluorescence spectra ($\lambda_{\text{ex}}=320 \text{ nm}$) of 1 ($2.0 \times 10^{-5} \text{ mol l}^{-1}$) in acetonitrile–water (20:80 v/v, pH 7.4) with increasing amounts of Hg^{2+} . Curves 1–12 correspond to 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0, 2.4, 3.0, $4.0 \times 10^{-5} \text{ mol l}^{-1}$ respectively. Inset showed fluorescence intensity area ($\lambda_{\text{ex}}=320 \text{ nm}$, $\lambda_{\text{em}}=350\text{--}500 \text{ nm}$) against the concentration of Hg^{2+}



Scheme 1 Hg^{2+} -induced cyclization of receptors 1 and 2



collected for the solution prepared in the same way like those for fluorescence studies.

Results and discussion

The fluorescence titration of 1 with Hg^{2+} was conducted in pH 7.4 water-acetonitrile (80/20 v/v) solution. The presence of Hg^{2+} in 1's aqueous solution induced a dramatic fluorescence increase. Figure 1 depicted the fluorescence spectral change of 1 with the increasing amounts of Hg^{2+} . The presence of 1 Eq Hg^{2+} gave a 73-fold enhancement in fluorescence intensity with respect to the metal-free solution. Meanwhile, the emission peak shifts from 383 to 388 nm. According to the references [28–33] the enhance-

ment in fluorescence intensity was probably associated with Hg^{2+} promoted desulfurization reaction as shown in Scheme 1. Owing to the high thiophilic characteristic of Hg^{2+} , 1,3,4-oxadiazoles were formed from thiosemicarbazides. The effects of temperature and time on the process were investigated. Five-minute bath warming in 60 °C water was optimal experiment condition. All experiments were performed under this condition.

The ESI mass and infrared spectroscopy provided further evidences for desulfurization reaction. The ESI mass spectra of 1 and the above reaction liquid were measured separately. The mass of 1's 1,3,4-oxadiazole derivative was calculated to be 280.13. A very clear and strong peak at $m/z=281.34$ which was attributed to $M+H^+$ was detected (as shown in Figs. S1 and S2). At the same time, the infrared spectra of 1

Fig. 2 Fluorescence spectra of 1- Hg^{2+} ($2.0 \times 10^{-5} \text{ mol l}^{-1}$) in the presence and absence of iodine ion without heating respectively. Inset plot indicated fluorescence spectral changes of 1- Hg^{2+} ($2.0 \times 10^{-5} \text{ mol l}^{-1}$) when iodine ion was added after the mixture was heated for 5 min in 60 °C water bath

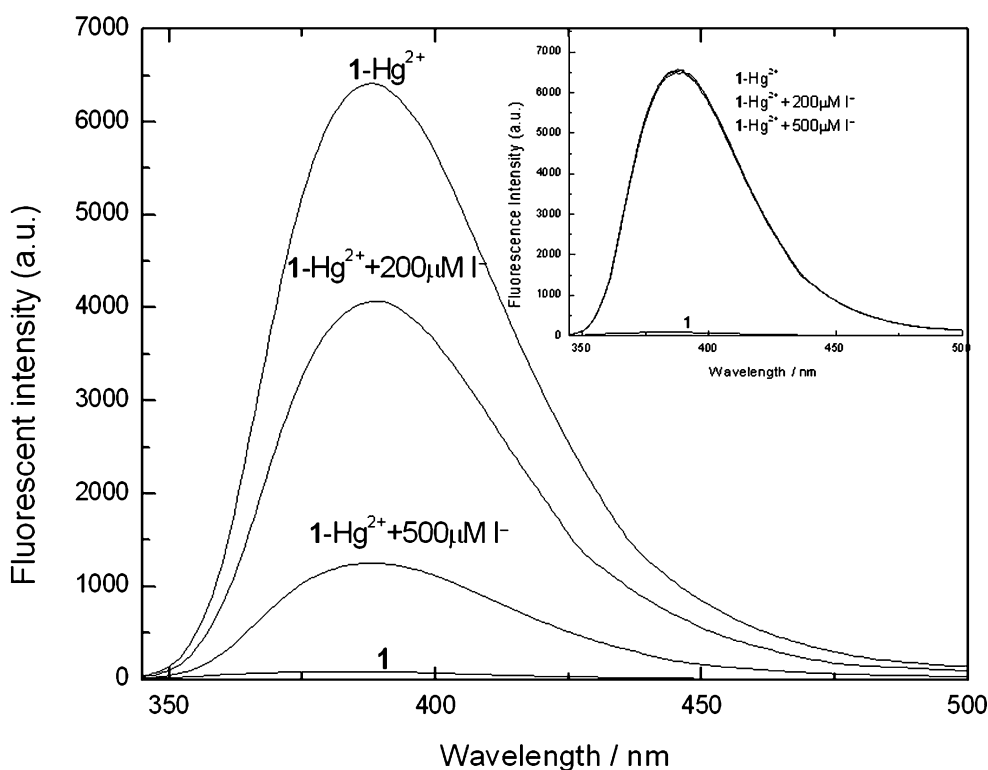
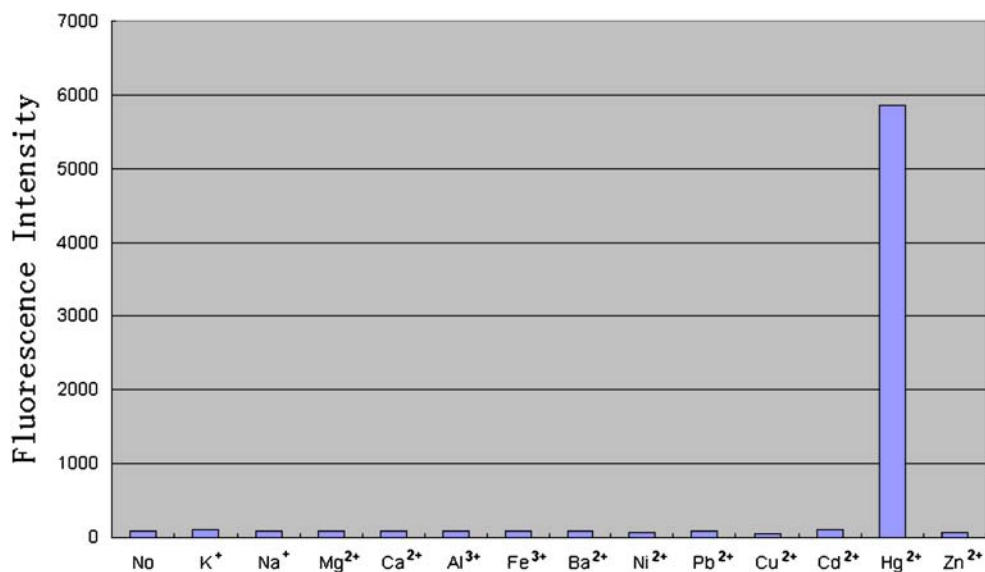


Fig. 3 The fluorescence intensity change of **1** ($2.0 \times 10^{-5} \text{ mol l}^{-1}$) at 388 nm with excitation wavelength set as 320 nm in the presence of 1 Eq metal ions in pH 7.4 aqueous solution



in the presence and absence of Hg^{2+} were also collected. The disappearance of $\gamma_{\text{C=O}}$ at $1,665 \text{ cm}^{-1}$ and the appearance of $\gamma_{\text{C-O}}$ indicated the cyclization reaction (as shown in Fig. S3).

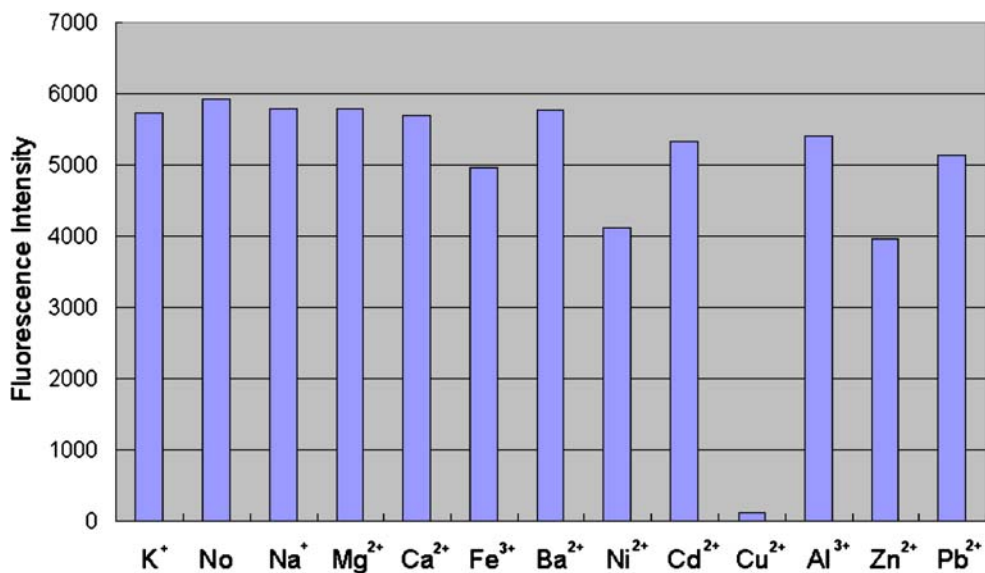
In order to prove the existence of 1-Hg^{2+} complex, the competing ion like iodine ion was added and the result was shown in Fig. 2. Without heating, the presence of the iodine ion induced fluorescence quenching due to the probable formation of HgI_2 and the dissociation of 1-Hg^{2+} . However, as shown in the inset plot, addition of iodine ion into the mixture of **1** and Hg^{2+} solution which has been heated for 5 min in 60°C water bath didn't result in any observable spectral change. Obviously, the heating speeded the desulfurization process.

According to the fluorescence titration, a good linear response between fluorescence intensity at 388 nm or

fluorescence intensity area from 350 to 500 nm and Hg^{2+} concentration was obtained ranging from 1.0×10^{-7} to $2.0 \times 10^{-5} \text{ mol l}^{-1}$. The limit of detection valued as three times of the standard deviation of the background noise was found to be $3.1 \times 10^{-8} \text{ mol l}^{-1}$, indicating a promising approach for simple and rapid tracking of trace mercury ions in biological or environmental system. Inset of Fig. 1 exhibited the change of the fluorescence intensity from 350 to 500 nm versus the concentration of Hg^{2+} and the fluorescence intensity kept constant after 1 Eq Hg^{2+} was added, which revealed that Hg^{2+} chemodosimeter had a 1:1 stoichiometry.

The fluorescence responses of **1** to various cations were illustrated in Fig. 3. It was clear that only the presence of Hg^{2+} led to a dramatic enhancement in fluorescence intensity. Upon addition of 1 Eq cations such as K^+ , Na^+ , Mg^{2+} , Ca^{2+} , Al^{3+} , Fe^{3+} , Ni^{2+} , Pb^{2+} , Cu^{2+} , observable

Fig. 4 Fluorescence intensity of **1** ($2.0 \times 10^{-5} \text{ mol l}^{-1}$) in the presence of mercury ion and competing ions



spectral profile and intensity changes didn't occur. As it has been previously reported, the presence of Zn^{2+} and Cd^{2+} led to a new emission at long wavelength with puny emission intensity change at short wavelength [34]. The results implied that 1 showed highly sensitive and selective response to Hg^{2+} . Despite its thiophilic nature, 10 Eq Ag^+ didn't quench the fluorescence owing to the existence of chloride ion. Comparative experiment performed in non chloride aqueous solution displayed that the presence of Ag^+ in 1's solution induced the similar result like that of Hg^{2+} .

The effects of coexisting ions on Hg^{2+} determination were also investigated and shown in Fig. 4. According to fluorescence intensity change which was less than $\pm 10\%$ with respect to intensity in the presence of Hg^{2+} , addition of 1000-fold K^+ , Na^+ , Ca^{2+} , Ba^{2+} , NH_4^+ , F^- , CH_3COO^- , SO_4^{2-} , ClO_3^- , $C_2O_4^{2-}$, and PO_4^{3-} , 500-fold Mg^{2+} , Br^- , $S_2O_3^{2-}$, and NO_3^- , 20-fold Al^{3+} and Pb^{2+} , 10-fold Cd^{2+} , 5-fold I^- , 0.5-fold Fe^{3+} , 0.1-fold Ni^{2+} and Zn^{2+} didn't interfere the determination of Hg^{2+} . Cu^{2+} caused fluorescence quenching and interfered the detection of Hg^{2+} .

The spectral property of receptor 2 was studied via fluorescence spectroscopy as well. Addition of Hg^{2+} into 2's solution also resulted in an enhancement of fluorescence intensity. However, upon the initial addition of Hg^{2+} , the enhancement in emission intensity was very weak. Dramatic increase of intensity was observed with increasing amounts of Hg^{2+} (shown in Fig. S4). Because on contrast to group phenyl in 1, group methyl in 2 is a good electron donor with less steric hindrance, 2- Hg^{2+} is more stable than 1- Hg^{2+} . The desulfurization reaction can easily occur for 1- Hg^{2+} than 2- Hg^{2+} .

Conclusions

A selective and sensitive fluorescent chemodosimeter for the determination of Hg^{2+} in aqueous solution was proposed. The fluorescence intensity was proportional to the Hg^{2+} concentration with the detection limit of 3.1×10^{-8} mol l^{-1} . It was promising for determining trace Hg^{2+} in environmental or biological samples. Hg^{2+} promoted desulfurization reaction led to the formation of a strongly fluorescent 1,3,4-oxadiazoles, which caused the fluorescence enhancement.

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