

Synthesis and Characterization of a Rapid and Highly Selective Fluorescent Hg²⁺ Sensor in Aqueous Media

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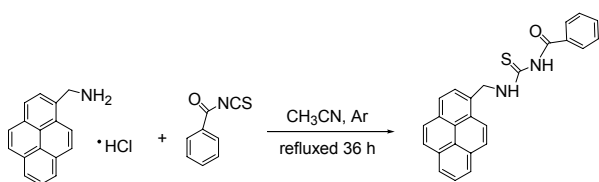
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Heavy metal ions pose risks to human health and environment.¹ Among the metal ions, Hg²⁺ is one of the most important toxic metal ions, because of its wide use in electrical equipment, catalysts, and paints, after which it is released into environment.² Thus, it is highly desirable to develop a Hg²⁺ sensor applicable for industrial waste sites and the environment. Among known metal ion sensors, fluorescent sensors have drawn much interest owing to their potential for on-site and real-time detection of toxic metal ions with low detection limits.^{1,3} A number of fluorescent Hg²⁺ sensors have been reported, including small molecules, whose quenching process is based on Hg²⁺ coordination.^{4,5} To enhance the sensitivity, however, it is essential to design a sensor that does not quench fluorescence upon metal ion recognition. Although there have been reports of a fluorescent turn-on Hg²⁺ sensor by several groups, still there are some drawbacks such as multiple synthesis steps from commercially available materials and low overall synthetic yield.⁶⁻¹⁰ Hence, it is desirable to develop new types of simple fluorescent sensors applicable in a variety of areas such as environmental monitoring and diagnostic analysis.

The pyrene is one of the most widely used fluorescent dyes in the design of fluorescent sensors for heavy metal ions.¹¹ Because of strong thiophilic affinity of Hg²⁺, carbamoyl thiourea derivatives have showed good binding property to Hg²⁺ in fluorescent sensors.^{12,13} But there are still some drawbacks including interference from other metal ion^{12,13} or irreversibility.¹⁴

In the present study, we report the synthesis and metal-binding properties of new pyrene-based Hg²⁺ fluorescent sensor **Py-1**. The fluorescent Hg²⁺ sensor **Py-1** was prepared in one step from commercially available 1-pyrenemethylamine hydrochloride and benzoyl isothiocyanate in high yield. (Scheme 1) We found that this turn-on sensor **Py-1** exhibits a rapid response and an extremely good selectivity toward Hg²⁺ compared with other metals in aqueous solution. We believe that it could have application in a variety of areas, such as environmental monitoring and diagnostic analysis.

Py-1 was prepared according to Scheme 1. Under argon,



Scheme 1. Synthesis of fluorescent sensor **Py-1**

a solution of 1-pyrenemethylamine hydrochloride (0.268 g, 1 mmol), benzoyl isothiocyanate (0.163 g, 1 mmol) in CH₃CN (20 mL) was refluxed for 36 h. Then 30 mL CHCl₃ was added, and the solution was filtered. The filtrate was removed by rotary evaporation to produce a yellow-orange solid. The crude product was purified by column chromatography (silica, CH₂Cl₂/hexane, 1/2) to give 0.296 g as yellow solid in 75% yield.¹⁵ The molecular structure of **Py-1** was confirmed by mass spectrometry and NMR. Fluorescence spectra were recorded with a FL-4500 fluorometer. Stock solutions (1.00 mM) of metal salts were prepared in H₂O/CH₃CN (1:1, v/v). Stock solutions of free **Py-1** (1.00 mM) were prepared in CH₃CN.

The effects of metal ion addition on the absorption and fluorescence properties of **Py-1** in CH₃CN/H₂O (1/1) were investigated to evaluate the metal ion binding properties of the sensor. We did not observe any changes in absorption spectra upon addition of metal ions except for Ag⁺. The fluorescence spectra of **Py-1** (c = 10⁻⁶ M) were recorded following 342 nm excitation. Figure 1 shows the fluorescence spectra of **Py-1** upon the addition of various metal ions, Ag⁺, Cd²⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Zn²⁺, and Hg²⁺. In the absence of metal ion, **Py-1** exhibited a very weak fluorescence intensity compared with that of pyrene owing to efficient photo-induced electron transfer from the metal binding site to the fluorescent unit. When Hg²⁺ was introduced to a 1 μM **Py-1**, a 23-fold fluorescence intensity enhancement was observed, a result of inhibition of the PET quenching pathway. On the other hand, little fluorescence enhancement

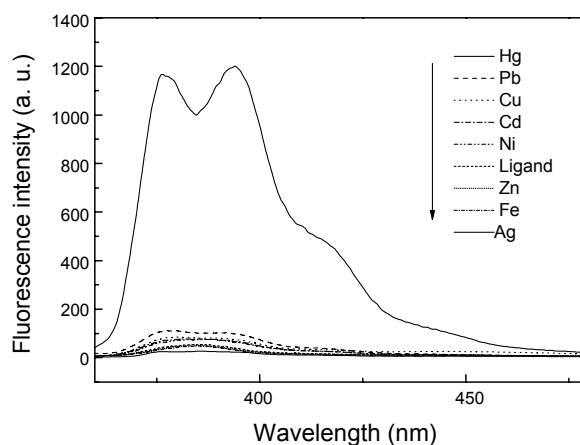


Figure 1. Fluorescence spectra of fluorescent sensor **Py-1** (1 μM) in H₂O/CH₃CN (1:1, v/v) in the presence of Hg²⁺, Ag⁺, Cd²⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Zn²⁺ (10 equiv).

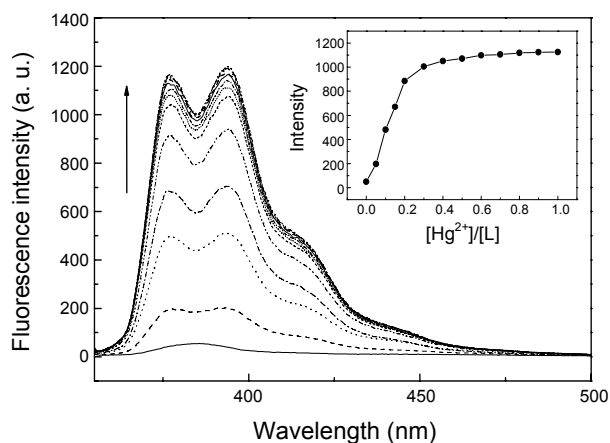


Figure 2. Fluorescence titration spectra of fluorescent sensor **Py-1** ($1 \mu\text{M}$) in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:1, v/v) upon addition of various amounts of Hg^{2+} (0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 equiv).

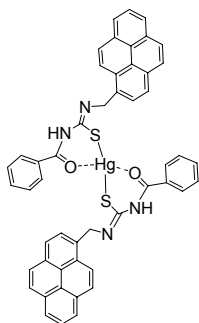


Figure 3. Proposed geometry of the complexes.

was observed for other metal ions. These results indicate that the fluorescence sensor **Py-1** exhibits a good selectivity toward Hg^{2+} over other metals in aqueous solutions. The fluorescence spectrum of **Py-1** in the presence of Hg^{2+} exhibits similar to that of pyrene monomer, indicating that there is no dimer formation upon Hg^{2+} ion complex.

Titration experiments were performed with $1.0 \mu\text{mol}$ solutions of fluorescent sensor **Py-1** and various concentrations of Hg^{2+} in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:1, v/v). Figure 2 illustrate the fluorescence titration spectra of **Py-1** ($1 \mu\text{M}$) upon addition of various amounts of Hg^{2+} (0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 equivalents). It can be seen that the fluorescence intensity increases continuously with increasing Hg^{2+} concentration. The inset shows the fluorescence titration profile of **Py-1** at 390 nm upon addition of Hg^{2+} . The maximum point appears at the mole fraction of 0.70 in a Job's plot, suggesting that the **Py-1** forms a 2:1 ligand-to-metal complex. Mass spectrometry data also confirmed the formation of 2:1 complex. To elucidate the binding mechanism of **Py-1**- Hg^{2+} complex, IR spectrum was measured in solid form. Upon addition of 10 equivalents of Hg^{2+} , the amide carbonyl band (1698 cm^{-1}) of **Py-1** shifts to a lower frequency (1633 cm^{-1}), indicating that the amide carbonyl participates in coordination with Hg^{2+} . In addition, ^1H NMR spectra also showed that the proton on thioamide was disappeared upon addition of Hg^{2+} , which indicate that the strong

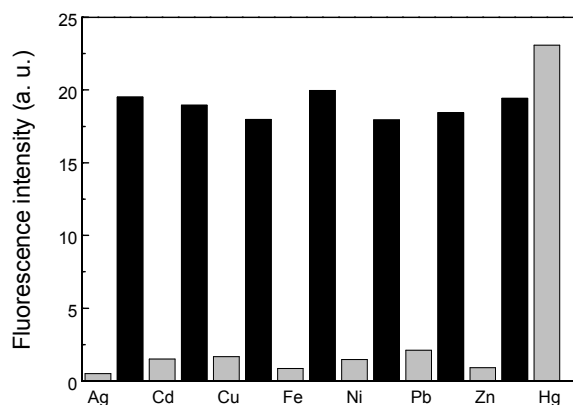


Figure 4. Selectivity and competition property of fluorescent sensor **Py-1** over the metal ions of interest. Gray bars: addition of 10 equiv of Ag^+ , Cd^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Hg^{2+} , to the solution; Black Bars: addition of 10 equiv of $\text{Ag}^+ + \text{Hg}^{2+}$, $\text{Cd}^{2+} + \text{Hg}^{2+}$, $\text{Cu}^{2+} + \text{Hg}^{2+}$, $\text{Fe}^{3+} + \text{Hg}^{2+}$, $\text{Ni}^{2+} + \text{Hg}^{2+}$, $\text{Pb}^{2+} + \text{Hg}^{2+}$, $\text{Zn}^{2+} + \text{Hg}^{2+}$, respectively in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:1, v/v).

interactions between Hg^{2+} and the sulfur atom of the thioamide group (Figure 3^{12,13}). To examine the reversibility of **Py-1**, excess of EDTA was added to the **Py-1**- Hg^{2+} complex that exhibited weak fluorescence intensity, demonstrating reversibility to free Hg^{2+} ion.

Figure 4 represents the selectivity and completion property of **Py-1** over the metal ions of interest (Gray bars: addition of 10 equivalents of Ag^+ , Cd^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Hg^{2+} , to the solution; Black bars: addition of 10 equiv of $\text{Ag}^+ + \text{Hg}^{2+}$, $\text{Cd}^{2+} + \text{Hg}^{2+}$, $\text{Cu}^{2+} + \text{Hg}^{2+}$, $\text{Fe}^{3+} + \text{Hg}^{2+}$, $\text{Ni}^{2+} + \text{Hg}^{2+}$, $\text{Pb}^{2+} + \text{Hg}^{2+}$, $\text{Zn}^{2+} + \text{Hg}^{2+}$, $\text{Hg}^{2+} + \text{Hg}^{2+}$, respectively, in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:1, v/v)). All data (F) were normalized with respect to the emission of the free dye (F_0). According to competition experiments, other metal ions did not show any interference with Hg^{2+} selectivity. Even addition of paramagnetic Ni^{2+} , Cu^{2+} , and Fe^{3+} , which are well-known fluorescence quencher, did little to quench fluorescence. These results demonstrate the applicability of the fluorescent sensor **Py-1** for Hg^{2+} specific sensing in the environment.

In conclusion, we have synthesized a pyrene-based fluorescent sensor, **Py-1**, and characterized its metal-binding properties. We found that fluorescent sensor **Py-1** exhibits a rapid response and an extremely good selectivity toward Hg^{2+} over other metal ions in aqueous solution.

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 - ¹H NMR (CDCl₃, 300 MHz) 5.60 (d, 2H, *J* = 3 Hz), 7.46 (t, 2H, *J* = 7.8 Hz), 7.58 (t, 1H, *J* = 7.5 Hz), 7.77 (d, 2H, *J* = 7.8 Hz), 8.00-8.11 (m, 4H), 8.16-8.22 (m, 4H), 8.30 (d, 1H, *J* = 9.3 Hz), 9.12 (brs, 1H), 11.12 (brs, 1H); ¹³C NMR (CDCl₃, 75 MHz) 48.3, 122.6, 124.6, 124.8, 125.0, 125.4, 125.5, 126.1, 127.2, 127.3, 127.7, 128.5, 129.0, 129.1, 129.2, 130.7, 131.2, 131.5, 133.5, 166.7, 179.5. HRMS (70 eV, EI): *m/z* calcd for C₂₅H₁₈N₂OS [M⁺] 394.1140, found 394.1135.