A highly selective pyrene based fluorescent sensor toward Hg^{2+} detection

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ABSTRACT

A new pyrene-containing fluorescent sensor has been synthesized from 2,3,3-trimethylindolenine. Spectroscopic and photophysical properties of sensor are presented. The large change in fluorescence intensity ($I/I_0 = 0.13$) at 381 nm and affinity to Hg^{2+} over other cations such as K$^+$, Na$^+$, Ca^{2+}, Mg^{2+}, Pb^{2+}, and Cu^{2+} make this compound a useful chemosensor for Hg^{2+} detection in hydrophilic media. The sensor (6.0×10^{-6} M) displays significant fluorescence quenching upon addition of Hg^{2+} in pH 7.4 HEPES buffer without excimer formation. Job's plot analysis shows the binding stoichiometry to be 2:1 (host/guest).

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1. Introduction

The annual release of mercury to the air by human activities (up to 4500 tons per annum) represents about 75% of the total yearly input of mercury into the environment from all sources.[1] This release results in significant impact on the environment and on human health.[2] As the main player in biological problems, Hg^{2+} is associated with human neurological diseases, and is especially harmful to prenatal life via Hg^{2+}-induced brain damage.[3] Because of the high toxicity of mercuric ion, even 5 ppb Hg^{2+} can result in significantly harmful effects. Therefore, developing rapid detection methods with a high sensitivity towards mercury has attracted great attention in past years.[4]

The widely used sensing method of fluorescent detection has been utilized for quantifying various molecules.[5] Compared to other approaches, fluorescent sensing shows excellent sensitivity, rapid response, and the ability to do the detection in a non-destructive manner.[6] Successful fluorescent sensors usually include three important parts, the fluorophore, a binding-recognition unit, and a signal conducting mechanism.[7] Organic dyes, luminescent metal complexes, and semiconductor quantum dots are usually used as the fluorophores due to their specific spectroscopic characteristics.[8] The binding-recognition unit specifically recognizes analytes and generates perturbation of the fluorophore following the binding to target molecules.[9] The signal conducting mechanism, such as Photoinduced Electron Transfer (PET), Intramolecular Charge Transfer (ICT), and Foster Resonance Energy Transfer (FRET) provides a connection between the binding event

and the reported signal.[10] Usually, the binding of an analyte on the molecular level results in spectrum change of fluorophore that is used as the signal to measure analyte.[11]

Recently, numerous fluorescent sensors have been reported for Hg^{2+} detection by using rhodamine,[12] 1,8-naphthalimide,[13] and cyanine.[14] These components provide fluorescence signals from the visible to near-infrared region of the electromagnetic spectrum. In addition to FRET and ICT,[15,16] chelation enhanced quenching (CHEQ) is also widely used as sensing mechanisms for sensor design..[17,18] These fluorescent sensors provide a powerful tool for monitoring Hg^{2+}.[19] However, despite lot of advantages, many sensors contain hydrophobic fluorophores that necessitate the addition of organic co-solvents to increase their solubility in aqueous media.[20] Poor solubility in a hydrophilic environment significantly hinders the application of Hg^{2+} sensors.[21]
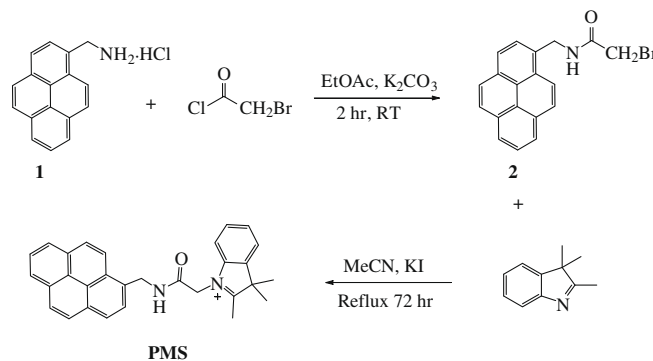
Scheme 1. Pyrene-based Mercury (II) Sensor (PMS) is prepared by a two-step reaction with 39% overall yield.

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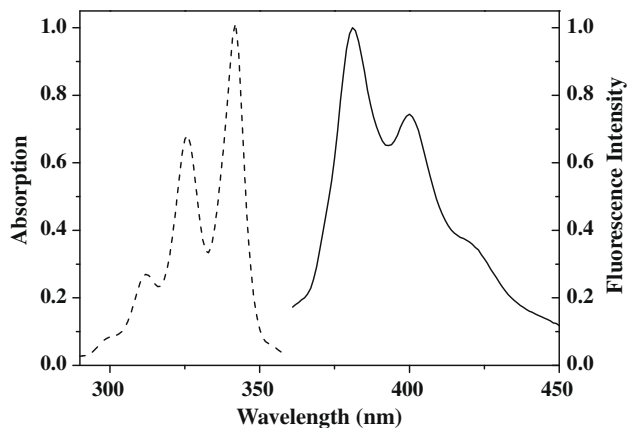 E-mail address: caoh1@unk.edu (H. Cao).

Figure 1. The absorption spectra (——) and fluorescence emission spectra (—) of PMS collected in pH 7.4 HEPES buffered (10 mM) aqueous solution.
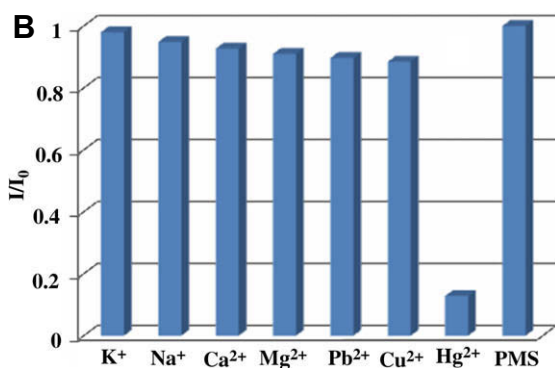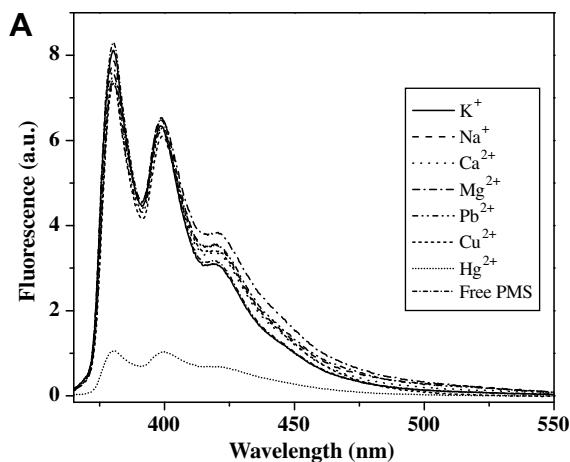

Figure 2. (A) Fluorescence spectra and (B) intensity change in of PMS (6.0×10^{-6} M) upon addition of perchlorate salts K^+, Na^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Cu^{2+} and Hg^{2+} (20 equiv) in pH 7.4 HEPES buffered (10 mM) aqueous solution at room temperature (λ_{em} = 340 nm).

Figure 3. (A) Fluorescence emission spectra and (B) fluorescence intensity of PMS (6.0×10^{-6} M) titrated with Hg^{2+} in pH 7.4 HEPES buffered (10 mM) aqueous solution (λ_{ex} = 340 nm, λ_{em} = 381 nm).

Figure 4. Job's plot of PMS with Hg^{2+} obtained by fluorescence measurements (λ_{ex} = 340 nm, λ_{em} = 381 nm). The total concentration of PMS and Hg^{2+} is 6.0×10^{-6} M (X = $[Hg^{2+}]/([Hg^{2+}]+[PMS])$).

In this Letter, we report a pyrene-based mercury (II) sensor (PMS) associated with chelation enhanced quenching (CHEQ) sensing mechanism. Due to the high detection sensitivity, pyrene was used as the fluorophore for PMS. An indolium was attached to pyrene for enhancing the solubility of PMS in aqueous solution. Our research involves the synthesis and examination of selectivity of PMS for Hg^{2+} against other physiologically relevant metal ions.

PMS was synthesized by a two-step reaction (Scheme 1). 1-Pyrenemethylamine hydrochloride was mixed with bromoacetyl
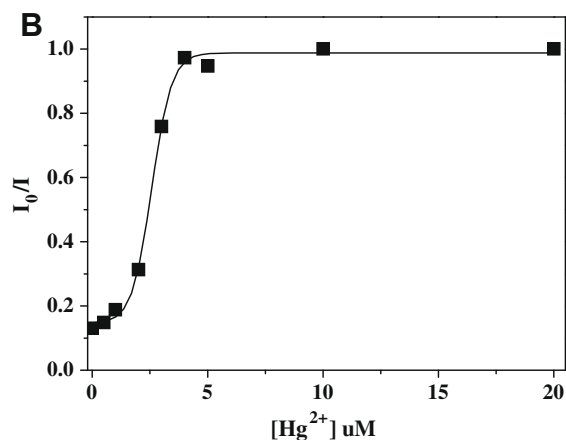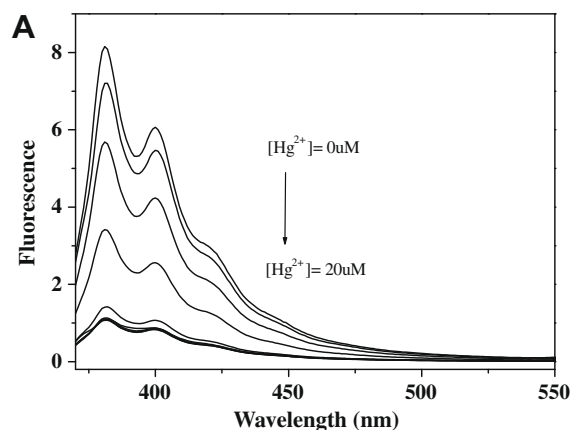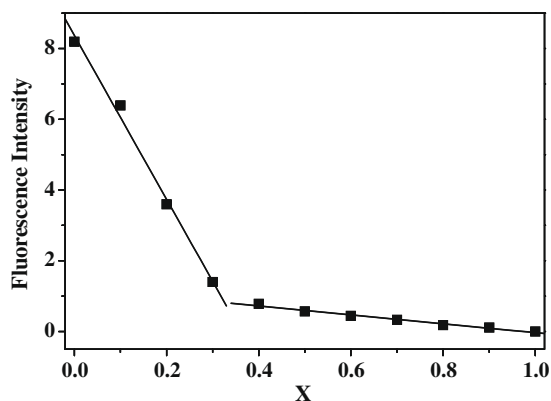
chloride in ethyl acetate and water mixture (1:1) for 2 h at room temperature to give compound **2** with 72% yield.[22] After 72 h refluxing with 2,3,3-trimethylindolenine and compound **2** in CH_3CN, PMS was obtained as a light brown powder in 54% yield (overall yield is 39%).[23]

The photophysical properties of PMS were investigated in pH 7.4 HEPES (10 mM) buffer. PMS displays a maximum absorption at 326 nm and 342 nm with distinct shoulders at 312 nm. The absorption bands are mostly due to a $\pi–\pi^*$ electron transition.
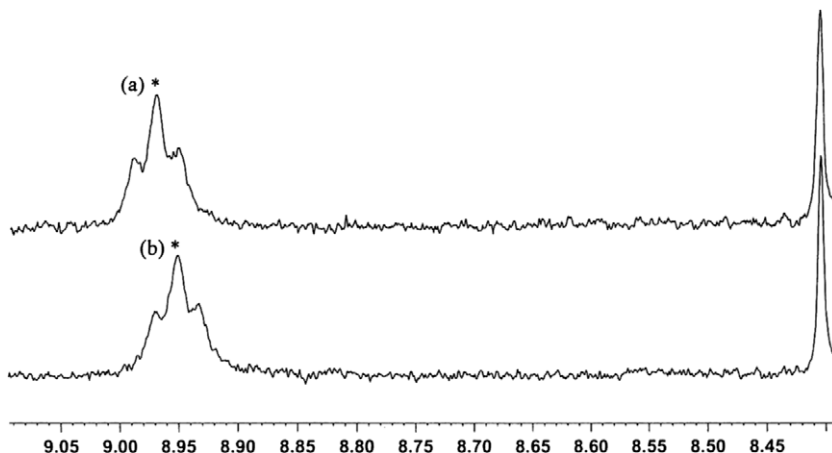
Figure 5. The ^{1}H NMR spectra for amide proton of PMS after (a) and before (b) addition of Hg^{2+}.

No change of the absorption spectrum were observed in presence of various metal ions. The emission spectrum of PMS showed two peaks at 381 nm at 400 nm (using 340 nm as the excitation wavelength), which are the typical bands of pyrene monomeric emission (Fig. 1). The fluorescence quantum yield of PMS (at 381 nm) was measured in pH 7.4 HEPES buffer using 9,10-diphenylanthracene as the reference ($\Phi_F = 0.37$).

The binding behavior of PMS (6.0×10^{-6} M) was investigated toward several physiologically relevant ions, including K$^+$, Na$^+$, Ca^{2+}, Mg^{2+}, Pb^{2+}, Cu^{2+}, and Hg^{2+} by using their perchlorate salts (1.2×10^{-4} M, 20 equiv) in pH 7.4 HEPES buffered (10 mM) aqueous solution. The fluorescence spectra were collected with an excitation wavelength at 340 nm. After incubation with these ions individually for 5 min at room temperature, PMS displayed fluorescence quenching for all ions (Fig. 2A). Among these metal ions, K$^+$, Na$^+$, Ca^{2+}, Mg^{2+}, and Pb^{2+} caused a slight decrease of fluorescence intensity (less than 10% at 381 nm) that indicated the weak binding between theses ions and PMS. In contrast, addition of Hg^{2+} led to significant fluorescence quenching of up to 87%. Moreover, Cu^{2+} also resulted in quenching (12%) but of much smaller scale than Hg^{2+} (Fig. 2B). These data are consistent to results reported by other groups.[24]

To examine the sensitivity towards Hg^{2+}, PMS (6.0×10^{-6} M) was titrated with Hg^{2+} ($0–2.0 \times 10^{-5}$ M) in pH 7.4 HEPES buffered aqueous solution (Fig. 3A). From the fluorescence titration profile of PMS versus [Hg^{2+}], the fluorescence quenching was detected in presence of Hg^{2+} as low as 5.0×10^{-7} M. Upon the continuous addition of Hg^{2+}, the emission spectrum was gradually quenched without wavelength shift. The maximum quenching was observed when the concentration of Hg^{2+} is 4.0×10^{-6} M that quenched 87% fluorescence of PMS at 381 nm (Fig. 3B). Based on these observations, the fluorescence quenching induced by Hg^{2+} was attributed to a heavy atom effect and was consistent to chelation enhanced quenching (CHEQ) sensing mechanism.[25] Using fluorescence

titration data, the association constant of PMS with Hg^{2+} was found to be 5.12×10^7 M^{-2}.[26]

In order to investigate the binding complex between PMS and Hg^{2+}, a fluorescence-based Job's plot ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 381$ nm) of fluorescence intensity against X ($X = [\text{Hg}^{2+}]/([\text{Hg}^{2+}]+[\text{PMS}])$) was created and shown in Figure 4. The maximum fluorescence intensity at $X = 0$ and the inflection point at $X = 0.33$ indicated the maximum amount of the PMS–Hg^{2+} complex was formed when the concentrations of Hg^{2+} and PMS were 1.98×10^{-6} M and 4.02×10^{-6} M, respectively. Therefore, this Job's plot clearly suggested that the PMS–Hg^{2+} complex exists with 2:1 stoichiometry.

^{1}H NMR spectra of free PMS and PMS–Hg^{2+} were also collected to elucidate the binding model of PMS with Hg^{2+} (Fig. 5). Compared to free PMS, the amide proton of PMS in presence of Hg^{2+} showed a down-field shift that indicated the nitrogen atom of amide involves in coordinating with Hg^{2+} by using its lone electron pair. This NMR data confirmed the formation of PMS–Hg^{2+} complex as obtained from fluorescence studies.

As a fluorogenic molecule, besides monomer emission wavelengths (I_M) at 370–400 nm, pyrene may also display an excimer emission wavelength (I_E) at 480 nm that is generated by a strong π–π interaction between two pyrenes.[27] Based on the ^{1}H NMR spectra and Job's plot analysis, PMS bond to Hg^{2+} to form a complex with stoichiometry PMS/Hg^{2+} = 2:1. The formation of PMS–Hg^{2+} complex may lead to two pyrene units closely approaching to give an intermolecular ground-state dimer. However, we did not observe any excimer emission of PMS–Hg^{2+} complex in presence of Hg^{2+} ($0–1.2 \times 10^{-4}$ M) in HEPES buffered solution. Fluorescence study indicated that the orientation of two pyrene moieties did not form efficient π–π interaction for excimer because of the excimer-unfavored PMS–Hg^{2+} complex (Fig. 6).

2. Conclusion

In conclusion, we have successfully synthesized a pyrene-based mercury(II) sensor (PMS) with high solubility in aqueous solution at physiological pH. The high affinity of PMS to Hg^{2+} is driven by the selective formation of a complex which causes 87% fluorescence intensity quenching at 381 nm. These data establish that PMS behaves as a highly sensitive fluorescent sensor for Hg^{2+} detection.
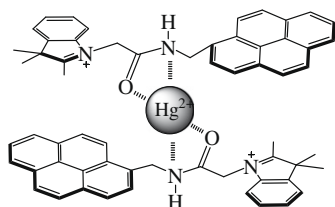
Acknowledgments

Figure 6. Complex formed by PMS and Hg^{2+} in 2:1 ratio without displaying excimer emission in pH 7.4 HEPES buffered solution.

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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.2010.04.100.

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22. *2-Bromo-N-(pyren-1-ylmethyl)acetamide* (**2**): 1-pyrenemethylamine hydrochloride (0.804 g, 3 mmol) mixed with potassium carbonate (1.68 g, 12 mmol) is suspended into a mixture of ethyl acetate (120 mL) and water (120 mL). Then, bromoacetyl chloride (7.07 g, 4.5 mmol) in ethyl acetate (10 mL) is added dropwise into the solution. After 2 h stirring at room temperature, the organic layer is isolated and dried by MgSO$_4$.[22] The ethyl acetate solvent is removed by rotary evaporation to give the crude product that is purified by column chromatography (silica, 220–400 mesh, hexane/EtOAc = 1:3 v/v). The product is isolated as a white powder **2** (0.76 g, 72%). ^{1}H NMR (300 MHz, DMSO) δ: 3.95 (s, 2H), 5.08 (d, 2H), 8.03–8.41 (m, 9H), 8.99 (t, J = 7.9 Hz, 1H); ^{13}C (75 MHz, DMSO) δ: 30.1, 123.7, 124.3, 124.5, 125.2, 125.7, 125.8, 126.8, 127.3, 127.6, 127.8, 128.3, 128.6, 130.7, 131.2, 132.7, 167.0. ESI/MS: m/z calcd for [M+H]$^+$ 353.02, found 353.04. Anal. Calcd for C$_{19}$H$_{14}$BrNO: C, 64.79; H, 4.01; N, 3.89. Found: C, 64.88; H, 4.07; N, 3.81.
23. *2,3,3-Trimethyl-1-(2-oxo-2-(pyren-1-ylmethylamino)ethyl)-3H-indolium* (**PMS**): To a solution of **2** (0.35 g, 1 mmol) in CH$_3$CN (10 mL), 2,3,3-trimethylindolenine (0.19 mL, 1.2 mmol) and KI (0.2 g, 1.2 mmol) are added and then refluxed for 72 h.[11] After reaction, the solvent (CH$_3$CN) is removed by rotary evaporation and the crude product is purified by column chromatography (silica, 220–400 mesh, CH$_2$Cl$_2$/EtOAc = 8:1 v/v) to afford **PMS** as a light brown powder (0.30 g, 54%). ^{1}H NMR (300 MHz, DMSO) δ: 1.22 (s, 6H), 2.20 (s, 3H), 4.19 (s, 2H), 5.08 (d, 2H), 7.11–7.48 (m, 4H), 8.00–8.39 (m, 9H), 8.90 (t, J = 7.9 Hz, 1H); ^{13}C (75 MHz, DMSO) δ: 15.5, 23.0, 41.3, 53.6, 119.0, 122.1, 123.6, 124.4, 125.2, 125.3, 126.8, 126.9, 127.1, 127.2, 128.1, 129.1, 129.2, 130.2, 130.7, 131.2, 132.0, 166.5, 188.1. ESI/MS: m/z calcd for [M+H]$^+$ 432.22, found 432.25. Anal. Calcd for C$_{30}$H$_{27}$IN$_2$O: C, 64.52; H, 4.87; N, 5.02. Found: C, 64.61; H, 4.81; N, 4.98.
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