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A Squaraine-Based Colorimetric and "Turn on" Fluorescent Sensor for Selective Detection of Hg^{2+} in an Aqueous Medium

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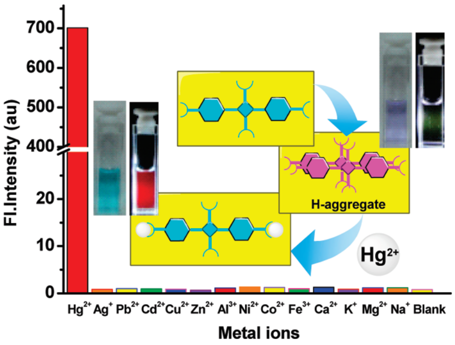
ABSTRACT



A novel squaraine-based chemosensor SQ-1 has been synthesized, and its sensing behavior toward various metal ions was investigated by UV−vis and fluorescence spectroscopies. In AcOH−H_2O (40:60, v/v) solution, Hg^{2+} ions coordinate with SQ-1 causing a deaggregation which induces a visual color and absorption spectral changes as well as strong fluorescence. In contrast, the addition of other metals (e.g., Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, and Ag^{+}) does not induce these changes at all. Thus SQ-1 is a specific Hg^{2+} sensing agent due to the inducing deaggregation of the dye molecule by Hg^{2+}.

Mercury is a deadly toxin to humans by causing cell dysfunction, that consequently leads to many health problems such as central nervous system defects and erethism as well as arrhythmia, cardiomyopathy, and kidney damage.[1] Thus, preparation of a highly specific, facile, and cost-effective mercury sensor, which is applicable to the common biological milieux, is an important goal.[2] Squaraine dyes are suitable for designing chemosensors because of their favorable optical properties which can be perturbed with many external factors, such as polarity, temperature, and pH of media and other additives.[3] A number of squaraine-based sensors have been synthesized, but only a few of them exhibit superior performance.[4] In most cases, their selectivity and sensitivity are not very promising.[5] Squaraine dyes involve a highly π-delocalized polymethine chromophore which consists of a central four-membered squaric ring 1,3-substituted by aniline moieties. In concentrated solution, they sometimes show a blue or a sharp red color due to the formation of the aggregates, while in the aqueous medium they tend to

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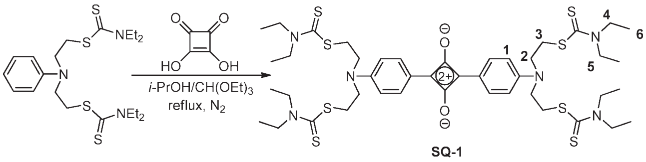
congregate and precipitate that can lead to dramatic color modulation and fluorescence quenching.[6] These phenomena have been successfully applied for designing sensors. For example, Ajayaghosh et al. designed a sensor based on the cation inducing aggregation of the dye. When squaraine dye podands bind with Ca^{2+}, they form folded or sandwich inclusion complexes, which are similar to the "H"-aggregates, leading to the solution color change from light-blue to intense purple and fluorescence quenching.[7]

Squaraines possess four potential binding sites: two oxides at the electron-deficient cyclobutene ring and two nitrogen atoms of anilino moieties; their coordination with metal ions can cause a color modulation of the dyes, i.e., absorption spectral changes. For example, a squaraine dye can consecutively bind two protons or metal ions causing a hypsochromic shift of the peak and then complete bleaching, respectively. This has been inferred reasonably as sequential coordination at the amine nitrogen atoms.[8] Martínez-Máñez and Rurack have reported a symmetrical and aggregation reversible squaraine dye hitched with a dithia-dioxa-aza crown, which is completely bleached on binding with an excess of Hg^{2+}; the monomer−aggregate absorption bands are reduced without affecting the band shape. In contrast, a similar binding with Ag^{+} shifts the color to green and also causes regaining of the monomer absorption band with a concomitant decrease in the dimer band. This is a nice sensor to discriminate Hg^{2+} over other metal ions except Ag^{+} in mixture samples but fails where Ag^{+} ions are present.[9]

Herein, we report a new squaraine dye that can specifically probe for the Hg^{2+} cation in complex aqueous samples containing common metal ions, even thiophillic cation Ag^{+} and Pb^{2+}, by means of colorimetric and fluorescent monitors. To the best of our knowledge, this is one of the very few squaraine-based "turn on" fluorescent sensors.[10]

Scheme 1. Synthesis of the Squaraine Dye **SQ-1**



The fact that dithiocarbamate (**DTC**) is an effective ligand for heavy metal ions[11] prompted us to select (phenylazanediyl)-bis-(ethane-2,1-diyl)-bis-diethyl-carbamodithioate as a binding arm to synthesize **SQ-1** by the reaction with squaric acid in *i*-PrOH with triethyl orthoformate as an *in situ* homogeneous absorbent as shown in Scheme 1 (also see pp S1−S4 in the Supporting Information). **SQ-1** is highly soluble in CH_2Cl_2, $CHCl_3$, and AcOH and also stable especially in AcOH without an absorption spectrum change over several months. It is known that squaraines are susceptible to nucleophilic attack which would be inhibited by acidic media. Our experiments show that in acetic acid **SQ-1** is robust showing good spectral reproducibility without the interference of the proton. It shows a fairly strong and symmetrical absorption band at 644 nm and an emission band at 661 nm, both of which arise from its donor−acceptor−donor type of charge transfer and the extensive conjugation structure. With increasing water % in the solution, the 644 nm absorption peak decreases and a new absorption peak at ca. 548 nm emerges, reaching a maximum at about 60% water (see Figure S5 on p S4 in the Supporting Information). The spectral change is interpreted, in analogy to the previous report,[12] as being the equilibrated formation of a "H"-aggregate (face-to-face dimer) and **SQ-1** monomer wherein the shorter new absorption arises from the "H"-aggregate at 60% water concentration

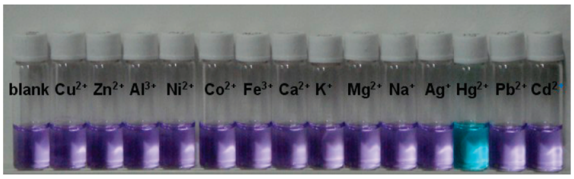


Figure 1. Color changes upon addition of different metal ions (10 equiv) to **SQ-1** (2 μM) in AcOH−H_2O (40:60, v/v) solution.

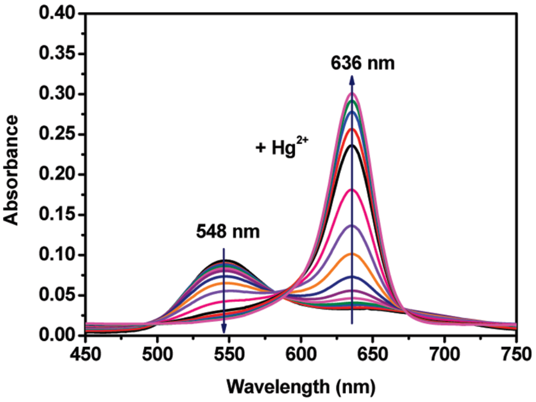


Figure 2. Absorption spectra of **SQ-1** (2 μM) upon addition of Hg^{2+} (0−27 μM) in AcOH−H_2O (40:60, v/v) solution.

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reaching the maximum; subsequent investigations were carried in AcOH−60%H_2O.

A solution of **SQ-1** in AcOH−60%H_2O showed no change in solution color or in both absorption and emission spectra upon addition of various metal ions (e.g., Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Fe^{3+}, Ag^+, Hg^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+}); the purple solution was dramatically changed to a blue color upon addition of Hg^{2+} ions (Figure 1). In the latter case, with increasing amounts of Hg^{2+} ions the absorption peak at 548 nm, ascribed to "H"-aggregates, diminished proportionally with the concomitant emergence of a new 636 nm peak with an isosbestic point at 587 nm as in Figure 2. These **SQ-1** solutions with an increasing concentration of Hg^{2+} ions also caused strong fluorescence in incremental strength up to approximately 700-fold (Figure 3). As shown in both figures, the addition of various other metal ions does not cause such a drastic change. The observations established that **SQ-1** exhibited a high specificity for qualitatively determining Hg^{2+} ions in the presence of various other metal ions. Cations such as K^+, Ca^{2+}, Cu^{2+}, Ag^+, and Pb^{2+} do not lead to any noticeable change in the fluorescence emission spectra (pp S5−S6, Supporting Information). It has a high potential for the design of a new chromogenic Hg^{2+} chemosensor for the quantitative determination of Hg^{2+} ions with multiple channels of measurements.

Preliminary studies on the detection limit were carried out using flurescence intensity titration against Hg^{2+} ion concentrations ranging from 0.13 to 0.63 μM, giving a good linear plot ($R = 0.994$, $k = 8.9 \times 10^7$ au/M). Therefore, the detection limit reached 7.1×10^{-9} M ($3\sigma/k$) (see p S8, Supporting Information), which was below the limit of Hg^{2+} in drinking water (2 ppb) set by the U.S. Environmental Protection Agency (EPA). The Job's plot showed a maximum value at the 0.66 mol fraction of Hg^{2+} ions, which is the signature of a 1:2 binding ratio between the dye and Hg^{2+} (p S8, Supporting Information).

It is well-known that squaraines typically possess a zwitterionic structure. The bond connecting the four-membered ring and a benzene ring has partial double bond character;[13] thus with the side arms upon coordination with a metal ion, the effect must be transmitted to the whole molecule from delocalization. Such effects were examined with ^1H NMR spectroscopy.[14] In $CDCl_3$, **SQ-1** shows a *para*-substitute benzene type double doublet at 7.09 and 8.42 ppm, four methylene signals as assigned in the

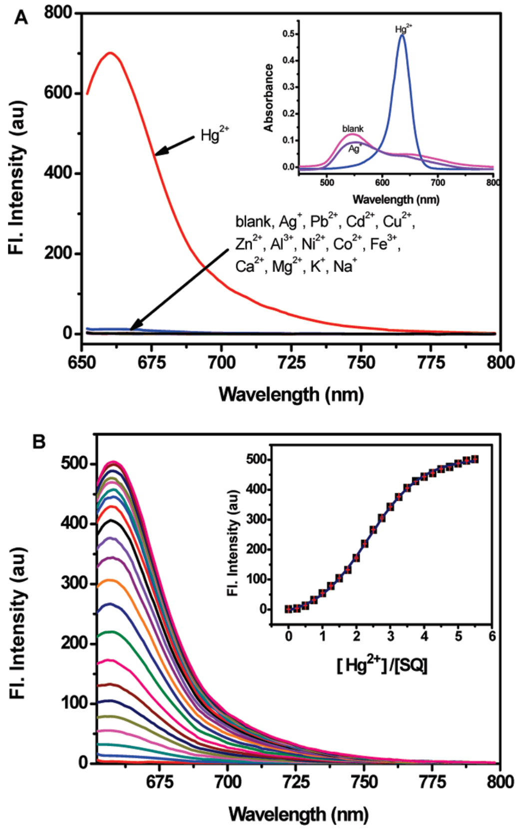

Figure 3. (A) Fluorescence spectrum of **SQ-1** (2 μM) in the presence of different metal ions (5 equiv) in AcOH−H_2O (40:60, v/v) solution. Inset: The corresponding absorption spectrum of **SQ-1** and in the presence of Ag^+ or Hg^{2+}. (B) Change in fluorescence intensity of **SQ-1** (2 μM) with increasing addition of Hg^{2+} (0−11 μM) in AcOH−H_2O (40:60, v/v) solution. Inset: The binding isotherm of **SQ-1** at 660 nm upon the addition of Hg^{2+}. Error bars for $n = 3$ ($\lambda_{ex} = 644$ nm, slit: 5 nm/5 nm, PMT Volts: 600).

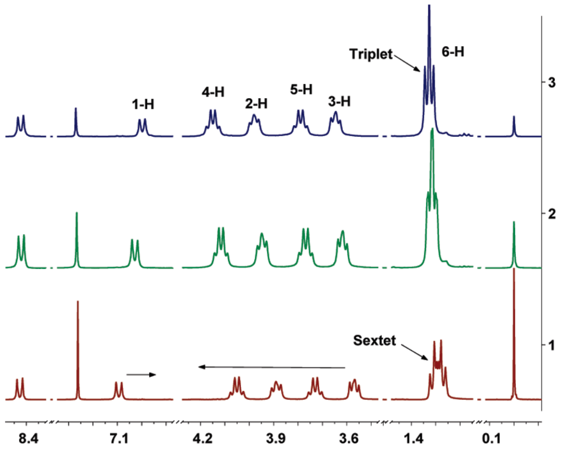

Figure 4. ^1H NMR spectrum of **SQ-1** in $CDCl_3$ with increasing concentration of $(CH_3COO)_2Hg$. The mole ratio of $[(CH_3COO)_2Hg]$ to [**SQ-1**] is (1) 0, (2) 1.0, and (3) 2.2, respectively.

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region of 3.50−4.20 ppm (Figure 4-1), and two overlapping triplets from different CH$_3$ of two nonequivalent *N*-ethyl groups located at 1.29 ppm (note the corresponding nonequivalence of the CH$_2$ groups). Upon addition of 1.0 and 2.2 equiv, respectively, of (CH$_3$COO)$_2$Hg to the solution, the 7.09 ppm aromatic signal shifts to a higher field ($\Delta\delta$ = 0.12) and all methylene signals shift to lower fields ($\Delta\delta$ = 0.12) incrementally. While the opposing shifts require further justification, the Hg^{2+} coordination with two DTC units is clearly demonstrated. It is interesting to note that upon coordination of both DTC groups while the methyl signals coalesce to a single triplet, the methylene signals remain nonequivalent with a substantial shift gap. It is desirable that the structure of the complex should be determined to clarify the magnetic environment of the *N*-ethyl groups.

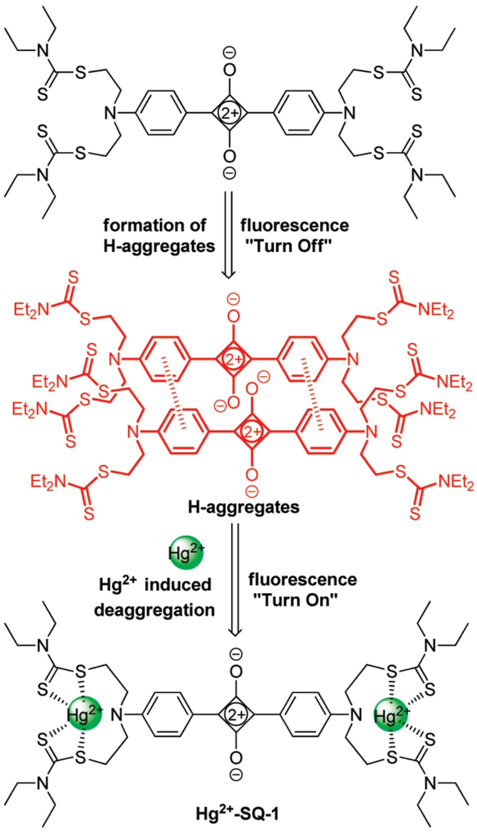

Figure 5. Process of sensing Hg^{2+} and the proposed binding model of "**Hg^{2+}-SQ-1**".

The steric hindrance of the coordinated Hg^{2+} complex with the **DTC** at the side arms might induce the deaggregation of the "H"-aggregates (Figure 5), resulting in the red shift of the absorption and the fluorescence enhancement. In addition, squaraine molecules possess two freely rotating alkyl chains as side arms; when they completely coordinated with Hg^{2+}, it could lead a reduction of the vibrational and rotational tendency to fluorophore, keeping the squaraine molecule a rigid structure and avoiding the system energy loss, which finally led to the fluorescence enhancement.

In conclusion, a symmetrical squaraine dye **SQ-1** was synthesized as a selective fluorescent and colorimetric sensor for Hg^{2+} in an aqueous medium. "Turn-on" fluorescence and color change were observed only for Hg^{2+}, which can be attributed to the "H"-aggregate deaggregation process. It was accompanied by a 700-fold fluorescence enhancement, and the detection limit was as low as 7.1 nM. Job's plot analysis supports the 1:2 binding mode of **SQ-1** and Hg^{2+}.

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Supporting Information Available. Synthetic details along with spectroscopic data of the squaraine dye **SQ-1**. This material is available free of charge via the Internet at http://pubs.acs.org.