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Dual-Mode Semisquaraine-Based Sensor for Selective Detection of Hg^{2+} in a Micellar Medium

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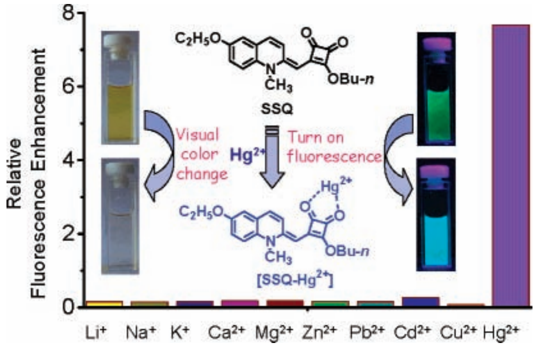
ABSTRACT



A novel chemosensor based on semisquaraine dye (SSQ) for selective detection of Hg^{2+} is described. SSQ is obtained in quantitative yields from the reaction between squaric acid and 6-ethoxy-2-quinaldinium iodide. SSQ in combination with surfactant shows a dual chromogenic and fluorogenic response selectively toward Hg^{2+} as compared to Li^+, Na^+, K^+, Ag^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, and Fe^{3+} due to the soft acid nature and size of the mercuric ion.

Detection of mercury and its compounds in the environment is of great interest because of their high toxicity, wide usage in a number of industrial processes, and biomagnification in the food chain.[1] Though several techniques are currently available for detecting mercury and its derivatives,[2] recently there has been a great interest in developing sensitive, real-time, and inexpensive methods for monitoring mercury contamination in water and the environment. In this respect, fluorosensors offer distinct advantages in terms of sensitivity and response time.[3] A number of fluorescence-based sensors have been reported recently including squaraines,[4] cholate foldamers,[5] calixarenes,[6] porphyrins,[7] quinolines,[8] fluorescein,[9] and oligonucleotides.[10] However, most of these systems exhibit fluorescence quenching when bound to metal ions[4a,5,6,10] and suffer from applicability in the aqueous medium due to

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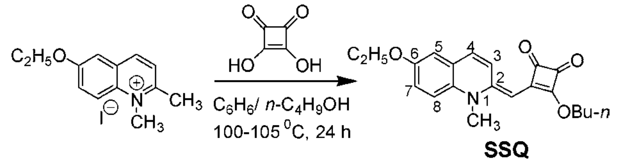
their poor solubility.[7] Progress in this area would require new strategies and simple molecular systems for the selective recognition of toxic metal ions and subsequent dual-mode[11] signaling of the event in aqueous solutions.

Recently, we have been interested in the synthesis of near-infrared absorbing squaraine dyes for applications in photodynamic therapy (PDT).[12] In this context, we have demonstrated that the quinaldinium salts substituted with electron-withdrawing substituents give the corresponding squaraine dyes in quantitative yields. However, the salts with electron-donating groups gave only semisquaraine dyes (SSQ) as the isolable products due to the reduced electrophilicity of the squaryl ring for further reaction with the enamine nucleophile.[13] We felt that SSQs which exhibit greater electron density at the squaryl moiety can, in principle, act as a novel bidentate ligand and form stable complexes with metal ions. Because these dyes possess favorable photophysical properties, the formation of such complexes should, in turn, alter the absorption and fluorescence intensity of the SSQ chromophore. Herein, we report a novel SSQ−surfactant system, which recognizes Hg^{2+} selectively in the aqueous medium and signals the event through visual color change and “turn on” fluorescence intensity. As far as we know, this is the first report which demonstrates the use of semisquaraine dye as a novel probe for the detection of metal ions.

As shown in Scheme 1, the reaction of 6-ethoxy-2-quinaldinium iodide with squaric acid in a mixture (1:1) of benzene and *n*-butanol gave semisquaraine dye (SSQ) in quantitative yields (experimental details, Figure S1, Supporting Information). SSQ exhibits good solubility in various organic solvents with absorption in the range 400−550 nm and molar extinction coefficients of $(3-5) \times 10^4$ M^{-1} cm^{-1}, depending on the nature of the solvent. For example, SSQ showed an absorption maximum at 505 nm and an emission band at 546 nm in acetone, whereas in methanol, it exhibited absorption and emission maxima at 478 and 538 nm, respectively. With a view to investigate the ability of SSQ as a bidentate ligand and thereby its potential use as a probe, we carried out its interactions with various metal ions under different conditions. When an aqueous solution of Hg^{2+} was added to SSQ in acetone, we observed a gradual change in color and a significant increase in fluorescence intensity of SSQ. In contrast, with the addition of other metal ions such as Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and Cu^{2+} under similar conditions, we observed negligible changes in the absorption and fluorescence properties, indicating thereby their negligible interactions with SSQ.

Scheme 1. Synthesis of the Semisquaraine Dye (SSQ)



Because SSQ showed unusual selectivity for Hg^{2+}, it was our goal to identify the ideal conditions for the detection event in aqueous solutions. In this context, we have investigated the interactions of SSQ with Hg^{2+} and other metal ions under different conditions including micellar media. We chose three surfactants such as cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and Triton X-100 (TX-100), which are representative examples of cationic, anionic, and neutral surfactants. Of all the conditions examined, it has been observed that a solvent system consisting of a mixture (9:1) of water and acetone containing SDS has been found to be very effective with respect to the stability of SSQ as well as to the selectivity and sensitivity of the metal ion binding event.

Figure 1A shows the changes in the absorption spectra of SSQ in a mixture (9:1) of water and acetone containing SDS (12 mM) with the increase in addition of Hg^{2+}. As the concentration of Hg^{2+} increased, we observed a regular decrease in the absorbance of SSQ at 472 nm with a

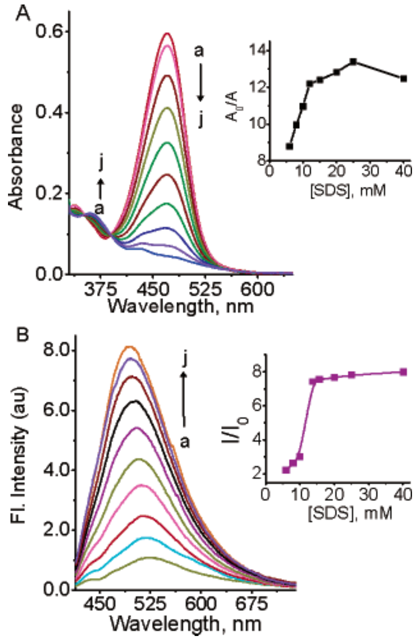


Figure 1. (A) Absorption and (B) the corresponding emission spectra of SSQ (20 μM) in a mixture (9:1) of water and acetone containing SDS (12 mM) with an increase in the addition of Hg^{2+} to (a) 0, (b) 2.2, (d) 6.6, (f) 11, (h) 15.4, and (j) 19.8 μM. Insets of A and B show the relative changes in the absorption and emission, respectively, of SSQ on complexation with Hg^{2+} as a function of [SDS]. Excitation wavelength = 392 nm.

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concomitant increase in absorbance at 360 nm (isosbestic point at 392 nm). Further addition of Hg^{2+} resulted in the complete disappearance of the absorbance at 472 nm, leading to a visual color change from a deep yellow to a colorless solution. Similarly, with an increase in the concentration of Hg^{2+}, we observed a gradual increase in fluorescence intensity of SSQ (Figure 1B). Addition of 19.8 μM Hg^{2+} gave ca. an 8-fold enhancement in fluorescence intensity along with a hypsochromic shift of 30 nm. This significant turn on intensity led to the visual observation of the change in fluorescence.[14] The absorption and fluorescence changes of SSQ in the presence of Hg^{2+} were analyzed through Job's and Benesi−Hildebrand plots (Figure 2), which gave a 1:1 stoichiometry for the complex between SSQ and Hg^{2+} with an association constant (K_{ass}) of $(4.0 \pm 0.1) \times 10^4$ M^{-1}.

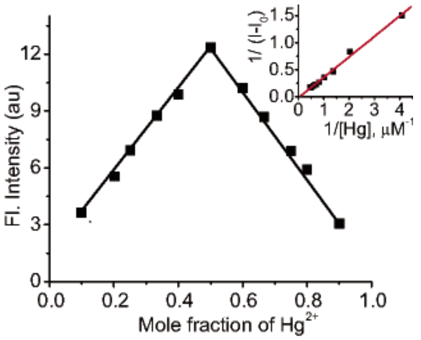

Figure 2. Job's plot for the complexation of SSQ with Hg^{2+} in a mixture (9:1) of water and acetone containing SDS (12 mM). Inset shows the Benesi−Hildebrand analysis of the emission changes for the complexation between SSQ and Hg^{2+}.

To investigate the effect of a micellar medium on the sensitivity of the assay, the detection of Hg^{2+} by SSQ has been examined by varying the concentration of SDS. Insets of Figures 1A and 1B show the relative changes in the absorption and fluorescence, respectively, of SSQ upon the addition of 1 equiv of Hg^{2+} at different concentrations of SDS. With an increase in concentration of SDS, the changes in the absorbance of SSQ upon addition of Hg^{2+} increased and reached a saturation at and above the critical micellar concentration (CMC)[12c,15] of SDS (Figures S2−S5, Supporting Information). Similarly, the enhancement in fluorescence intensity of the SSQ−Hg^{2+} complex is found to increase with an increase in concentration of SDS. However, the relative changes observed in the fluorescence intensity are found to be significantly higher than that of the absorption changes. Thus, in the presence of 6 mM SDS, we observed ca. a 2-fold enhancement in fluorescence intensity of SSQ upon binding with Hg^{2+}, and it reached a saturation with a maximum enhancement of ca. 8-fold at 12 mM SDS.

To confirm the nature of the complexation between SSQ and Hg^{2+}, the ^{1}H NMR spectrum of SSQ was recorded in the absence and presence of different concentrations of Hg^{2+} (Figure 3). The ^{1}H NMR spectrum of SSQ in $CDCl_3$ showed five aromatic protons as multiplets in the region between δ 7 and 9.5, whereas the olefinic and N-methyl protons appeared as singlets at δ 6.1 and 4.0, respectively. With an increase in the addition of Hg^{2+}, the aromatic proton at δ 9.45 (3-H, Scheme 1) showed a regular upfield shift ($\Delta\delta$ = 0.55 at 1 equiv of Hg^{2+}), and the other aromatic protons (4-H, 7-H, and 8-H) and the olefinic and N-methyl protons exhibited downfield shifts in the range $\Delta\delta = 0.35-0.6$ (Figure 3b−d). However, the aromatic proton such as 5-H and the aliphatic protons showed relatively negligible changes in their chemical shifts.

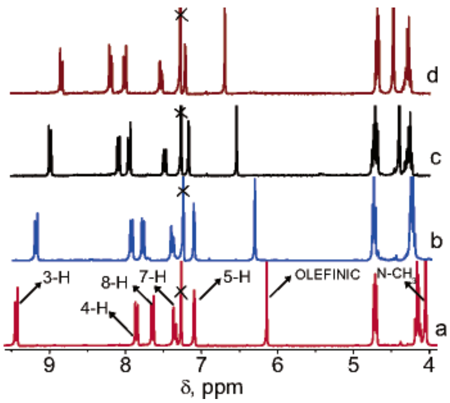

Figure 3. ^{1}H NMR spectrum of SSQ in $CDCl_3$ with increasing concentration of Hg^{2+} (in CD_3CN). The mole ratio of [Hg^{2+}] to [SSQ] is (a) 0, (b) 0.36, (c) 0.76, and (d) 1.0, respectively.

Interestingly, with the addition of 1 equiv of Hg^{2+}, the changes in the chemical shifts of these protons reached a saturation, confirming thereby the 1:1 stoichiometry for the complex between SSQ and Hg^{2+}. The significant upfield shift of aromatic proton 3-H and the downfield shifts observed for the other aromatic, olefinic, and N-methyl protons clearly indicate the involvement of two carbonyl groups of the squaryl moiety of SSQ in the complexation with Hg^{2+}.

The involvement of carbonyl groups of SSQ in the complexation was further confirmed through the analysis of infrared spectrum (FTIR) of SSQ in the presence and absence of Hg^{2+} (Figure S6, Supporting Information). The characteristic carbonyl stretching frequencies of SSQ appeared at 1766 and 1602 cm^{-1}. However, in the SSQ−Hg(II) complex, we observed a band at 1739 cm^{-1} instead of at 1766 cm^{-1}, and the band at 1602 cm^{-1} merged with that at 1570 cm^{-1} to give a single band at 1598 cm^{-1}, indicating that the binding with Hg^{2+} occurs at the two carbonyl groups of the squaryl moiety of SSQ.[16]

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(14) The complexation between SSQ and Hg(II) was found to be reversible in acetone with the addition of EDTA, but in a mixture (9:1) of water and acetone containing 12 mM SDS, it was irreversible due to the encapsulation of the SSQ−Hg(II) complex in the micellar medium.

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To demonstrate the selectivity of SSQ for Hg^{2}, we have investigated the interactions of SSQ with other environmentally important metal ions such as Li^{+}, Na^{+}, K^{+}, Ag^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, and Fe^{3+} in a mixture (9:1) of water and acetone containing 12 mM SDS. Figure 4 shows the relative changes in the fluorescence intensity of SSQ with the addition of 1 equiv of different metal ions. As can be seen from Figure 4, the addition of these metal ions showed negligible changes in the absorption and fluorescence emission of SSQ (Figures S7−S12, Supporting Information). The selectivity of SSQ toward Hg^{2+} can be observed visually because the deep yellow color and the fluorescence intensity of SSQ remained unchanged upon the addition of these metal ions, whereas with Hg^{2+}, we observed visual color change as well as turn on fluorescence intensity (Figure 4). Further, the presence of equimolar concentrations of these metal ions in the sample showed negligible influence in the sensitivity of SSQ for Hg^{2+} detection in aqueous solutions.

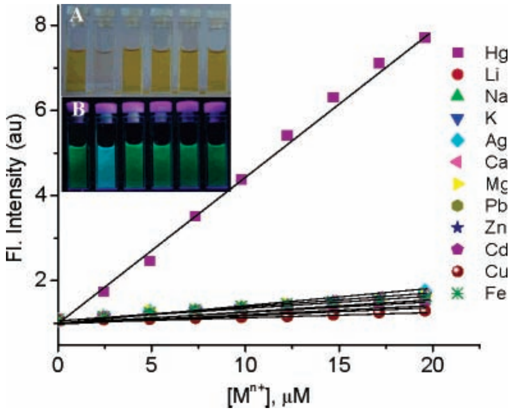

Figure 4. Fluorescence response of SSQ toward various metal ions in a mixture (9:1) of water and acetone containing SDS (12 mM). Inset shows visual changes in (A) absorption and (B) fluorescence of SSQ on adding various metal ions. From left to right: SSQ alone, plus metals ions Hg^{2+}, Na^{+}, Mg^{2+}, Pb^{2+}, Cd^{2+}.

Theoretical calculations have been carried out to understand the nature of the bonding between SSQ and Hg^{2+} (Figure 5).[17] The electrostatic potential map of SSQ showed the availability of a lone pair of electrons at the carbonyl oxygen atoms, and the distance between the two carbonyl oxygen atoms is found to be 3.3 Å. The selectivity of SSQ toward Hg^{2+} can be attributed to the soft acid nature[18] as well as the size of the mercuric ion because the fixed distance of 3.3 Å between the two carbonyl groups of the squaryl moiety can ideally accommodate a mercuric ion with a diameter of 2.2 Å. By taking into account the binding of SSQ with the metal ion, we propose that these dye molecules distribute within the micellar structure but close to the surface near the polar head groups. In such a situation, the hydrophobicity rendered by the aromatic moieties can be overcome by the hydrophilicity of the carbonyl oxygen atoms. This would enhance the affinity of the dye molecules toward polar ends so that stable complex formation between SSQ and Hg^{2+} could occur at the interface involving water molecules as other coordinates. The observed significant signaling efficiency of SSQ for Hg^{2+} in the presence of SDS as compared to the aqueous medium could be attributed to the microencapsulation of the complex. This results in the decrease in rotational as well as translational freedom of the excited state of the complex leading to the significant fluorescence enhancement in the presence of the surfactant.

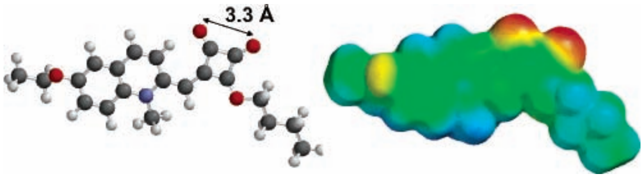

Figure 5. Geometry-optimized structure and the electrostatic potential map of SSQ.

In conclusion, we have designed and demonstrated the use of SSQ as a novel bidentate ligand and dual-mode sensor for the detection of Hg^{2+} in aqueous solutions. SSQ displayed unusual selectivity for the mercuric ion due to the soft acid nature and size of the metal ion and signals the event through color change and turn on fluorescence intensity. Further studies are in progress to understand the effect of various substituents in the recognition and to develop SSQ-based sensors for important metal ions.

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Supporting Information Available: Synthesis of SSQ and Figures S1−S12 showing the changes in IR, absorption, and fluorescence spectra of SSQ in the presence of various metal ions and SDS. This material is available free of charge via the Internet at http://pubs.acs.org.

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[17] All geometries are optimized using semiempirical AM1 calculations. DFT B3LYP/6-31.G* single-point energy calculations were used to obtain the electrostatic potential surfaces using Titan (Wavefuntion, Inc.). The color at each point on these surfaces reflects the interaction energy between a positive test charge at that point. Red indicates an attractive potential, whereas blue represents a repulsive potential. The areas of red therefore indicate a “negative” region, and yellow/green indicates a more neutral or “positive” region, depending on how blueish the color is.

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