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Detection of Aqueous Mercuric Ion with a Structurally Simple 8-Hydroxyquinoline Derived ON-OFF Fluorosensor

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Selective ON-OFF switching of an 8-hydroxyquinoline-derived fluorosensor was observed in the presence of Hg(II). The sensor can be easily synthesized in a straightforward two step procedure, allowing for determination of Hg(II) in pure unbuffered water. The detection limit of the sensor in water at 20 °C was determined to be 2.6 μM Hg(II).

Introduction

Research surrounding molecular devices that act as optical switches upon interaction with target analytes has increased greatly in the last decades.[1] A significant subset of these investigations deals with 8-hydroxyquinoline (8-HQ) and its derivatives, which has found much usage in the synthesis of molecular probes that exhibit a change in fluorescence emission upon coordination to metal cations such as Zn(II),[2] Cu(II),[3] Hg(II),[4] and others[5] of environmental and physiological significance. 8-HQ is desirable as a fluorophore and binding moiety because of the significant enhancement of fluorescence that is known to occur when the excited state intramolecular proton transfer (ESIPT)[6] between the hydroxy group and quinoline nitrogen is suppressed upon binding of a metal cation. For this reason, the vast majority of probes based on 8-HQ rely on OFF-ON fluorescence switching to signal the presence of analytes.

O-Benzyl ethers of 8-HQ, which are known to be highly fluorescent, have been successfully used as sensitizing antennae for promoting the luminescence of Eu(III) in various complexes,[7] but their application as fluorophores in optical switches is uncommon, presumably because further enhancement of fluorescence is unlikely in the context of an OFF-ON probe.

However, the high fluorescence of 8-*O*-substituted derivatives could be utilized in the construction of sensors that function in an ON-OFF manner, where fluorescence is quenched upon analyte addition. In addition, the use of a strongly fluorescent probe may allow for its application in aqueous environments where a large degree of solvent relaxation is possible. A recent comprehensive review[8] of the literature concerning optical detection of mercuric ion indicated only five examples[9] of ON-OFF probes functioning in water, none of which were based on the popular 8-HQ moiety. Clearly, contributions to this small population of aqueous ON-OFF sensors are necessary to extend the important realm of water-soluble probes. In this paper we report the two-step synthesis of a 8-benzyloxyquinoline-based ester fluorophore (BnOQE, **3**) that exhibits selective ON-OFF switching in the presence of Hg(II) in pure unbuffered aqueous solution.

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Experimental Section

General Techniques. All reagents and solvents used were of ACS grade or higher. Metal salts used in titrations were added as aqueous solutions in 18 MΩ ultrapure water of their corresponding nitrate salts, except for Fe(II) which was added as its sulfate salt. No effort was made to exclude oxygen. UV−visible measurements were acquired on a Thermo Scientific Evolution 300. Fluorescence spectra were performed on a Perkin-Elmer LS-55 luminescence spectrometer ($\lambda_{ex} = 303$ nm with 5.0 nm emission and excitation slit widths). NMR spectroscopy was performed in $CDCl_3$ using a Varian Mercury 200 MHz instrument. FT-IR measurements were made using a Nicolet Avatar 360. Mass spectra were recorded on a Varian 3900/Saturn 2100T GC/MS instrument.

Methyl 8-Hydroxyquinoline-2-carboxylate (2). To a stirred solution of 555 mg (2.93 mmol) 8-hydroxyquinoline-2-carboxylic acid in 20 mL of methanol was added 5 drops of concentrated sulfuric acid, and the solution was heated to reflux and allowed to stir overnight. The mixture was then evaporated under reduced pressure, and the residue taken up with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give 502 mg (2.47 mmol, 84%) of **2** as light yellow solid (mp = 99−101 °C) in need of no further purification. ^{1}**HNMR** (200 MHz, $CDCl_3$) δ 3.99 (s, 3H); 7.19 (dd, J = 1.2, 7.6 Hz, 1H); 7.30 (dd, J = 1.2, 7.6 Hz, 1H); 7.49 (m, 1H); 8.07 (d, J = 8.6 Hz, 1H); 8.19 (d, J = 8.6 Hz, 1H). ^{13}**CNMR** (50 MHz, $CDCl_3$) δ 53.0, 111.1, 117.7, 121.6, 129.7, 130.3, 137.4, 138.1, 145.2, 153.1, 165.3. **FTIR** ($CDCl_3$ film) 3415 (broad), 2956, 2859, 1729. **EIMS** (m/z) 203 (100%, M^+); 144 (10%, M-CO_2CH_3).

Methyl 8-Benzyloxyquinoline-2-carboxylate (3, BnOQE). To a stirred solution of methyl 8-hydroxyquinoline-2-carboxylate (502 mg, 2.47 mmol) in 15 mL of acetone was added benzyl bromide (0.32 mL, 2.7 mmol, 1.1 equiv) and potassium carbonate (677 mg, 4.9 mmol, 2.0 equiv). The solution was refluxed overnight before being filtered over Celite and concentrated under reduced pressure to give 703 mg (2.24 mmol, 91%) of crude BnOQE as a yellow solid. BnOQE used for fluorescence experiments was purified by column chromatography on silica gel (20% MeOH/CH_2Cl_2 (v/v)) and was isolated as colorless plate-like crystals (mp = 91−93 °C) upon evaporation. ^{1}**HNMR** (200 MHz, $CDCl_3$) δ 4.04 (s, 3H); 5.45 (s, 2H); 7.10 (dd, J = 1.8, 7.0 Hz, 1H); 7.41 (m, 7H); 8.20 (d, J = 8.6 Hz, 1H); 8.27 (d, J = 8.6 Hz, 1H). ^{13}**CNMR** (50 MHz, $CDCl_3$) δ 53.0, 71.1, 110.8, 119.6, 121.5, 127.1, 127.8, 128.5, 129.0, 130.7, 136.7, 137.1, 139.8, 146.7, 155.1, 166.1. **FTIR** ($CDCl_3$ film) 3159, 3073, 2951, 1723, 1470, 1336. **EIMS** (m/z) 293 (100%, M^+).

Results and Discussion

The synthesis of BnOQE proceeded smoothly (Scheme 1) starting with Fischer esterification of commercially available 8-hydroxyquinoline-2-carboxylic acid (**1**) in methanol to afford the corresponding methyl ester **2** in 84% yield. Alkylation using benzyl bromide/K_2CO_3 in acetone held at reflux overnight produced the 8-benzyloxy methyl ester **3** in 91% yield, resulting in 76% overall yield for the two-step sequence.

With the synthesis complete, the optical properties of BnOQE in the presence of commonly occurring metal cations were investigated. Evidence for ion interaction with the chromophore was first sought using UV−visible spectroscopy. One equivalent of an aqueous stock solution of the metal nitrate salts were added to a 10^{-4} M solution of BnOQE in pure water. No significant changes in the location of absorption maxima were detected upon addition of equimolar amounts of Ag(I), Na(I), Ca(II), Cd(II), Cu(II), Fe(II), Mg(II), Mn(II), Pb(II), Zn(II), or Al(III)[‡]. Upon addition of Hg(II), a marked red-shift in the local absorption maximum from 253 to 262 nm indicated significant interaction of Hg(II) with the quinoline π-system (Figure 1). Since mercuric ion was the sole species that induced a red shift in our chromophore, we proceeded to investigate the fluorescence behavior of BnOQE.

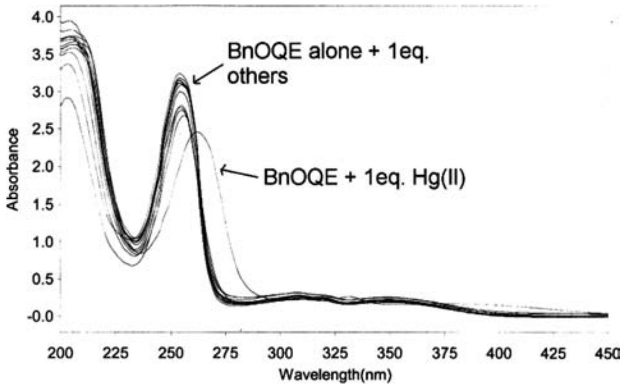
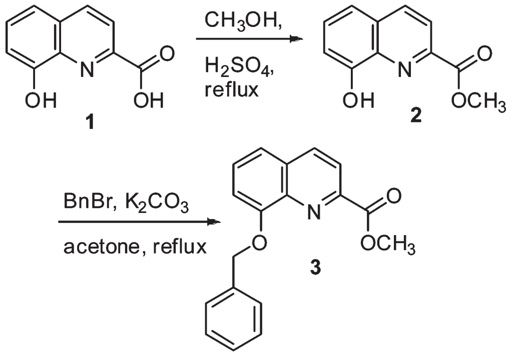


Figure 1. UV−visible spectra of BnOQE (10^{-4} M in H_2O) in the presence of equimolar Ag(I), Na(I), Ca(II), Cd(II), Cu(II), Fe(II), Mg(II), Mn(II), Pb(II), Zn(II), Al(III), and Hg(II).

Scheme 1. Synthesis of BnOQE (**3**)



An investigation of the fluorescence emission of BnOQE over a range of pH was carried out. For BnOQE to be suitable as a probe for the rapid monitoring of aqueous mercuric ion in environmental or biological settings, the fluorescence emission should be resistant to changes in pH that may occur in unbuffered natural systems or in the analysis of samples from mildly acidic and/or basic environments. The changes in the blue-green emission maxima located at 487 nm were monitored at solution pH values ranging from 1 to 13 (Figure 2).

While emission at 487 nm was greatly reduced in strongly acidic and basic media, no substantial change in the behavior of the fluorophore was observed upon measuring emission at a range of pH from 3 to 11. Uniform activity over such a wide range of pH makes BnOQE applicable for the analysis of environmental samples that would occur well within this extended range of pH, or for use in unbuffered media.

With this data in hand, we proceeded to explore the fluorescent properties of BnOQE in the presence of mercuric ion. Titration of a 25 μM aqueous solution of BnOQE with increasing amounts of Hg(II) showed continuous fluorescence quenching of the blue-green fluorescence

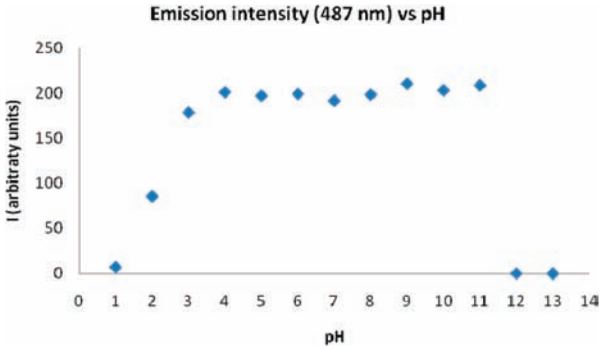


Figure 2. Fluorescence intensity at 487 nm recorded at various pHs. [BnOQE] = 25.0 μM in H_2O, λ_{ex} = 303 nm.

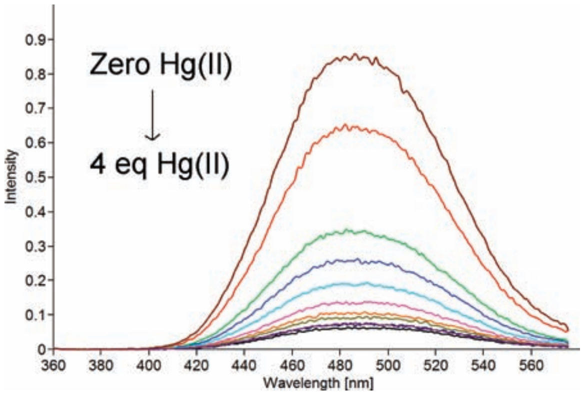


Figure 3. Fluorescence (in arbitrary units) titration of BnOQE with Hg (II). [BnOQE] = 25.0 μM in H_2O, λ_{ex} = 303 nm.

(λ_{max} = 487 nm) until the fluorophore was turned off by a molar excess of Hg(II) (Figure 3). The 100 μM Hg(II) solution that resulted from the titration had a pH of 5.2, which confirms that none of the observed fluorescence quenching is due to the acidification of the sample.

The fluorescence of BnOQE was effectively quenched by the addition of 1 equiv of Hg(II). No further quenching was observed upon the addition of molar excesses of Hg(II) to the system.

Coordination to Hg(II) as indicated by the UV−vis experiments should produce static fluorescence quenching through complex formation resulting in a non-emissive excited state.[10] This was readily observed by performing fluorescence titrations at both 20 and 40 °C.

Increased temperature will result in the decreased stability of the complex, and thus a smaller value of the static quenching constant (K_s). From the Stern−Volmer plots of I_0/I vs [Hg(II)], the calculated value[11] of K_s at elevated temperature was nearly 30× smaller (1.12×10^{-1} M^{-1} at 20 °C vs $K_s = 4.0 \times 10^{-3}$ M^{-1} at 40 °C) (Figure 4). To determine the detection limit of the sensor,[12] a linear regression curve was fit to the titration data obtained at 20 °C, and the point at which the line crossed the concentration axis was taken to be the detection limit and was equal to 2.6 μM (520 ppb) mercuric ion.

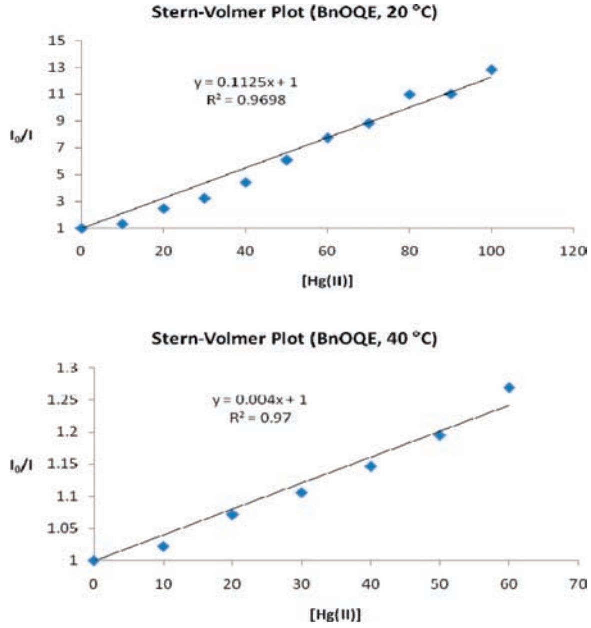


Figure 4. Stern−Volmer plots of I_0/I vs [Hg(II)] (μM) performed at 20 and 40 °C, [BnOQE] = 25.0 μM in H_2O, λ_{ex} = 303 nm.

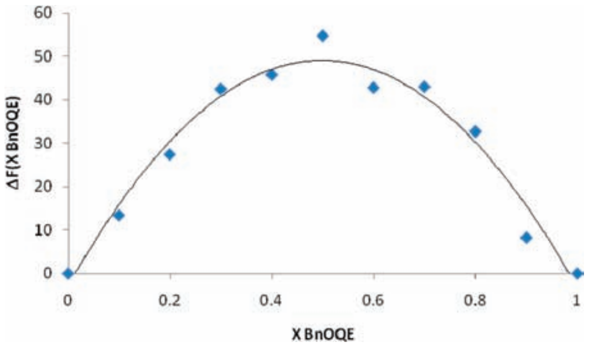


Figure 5. Job plot of changes in fluorescence intensity at varying mole ratios of BnOQE and Hg(II). [BnOQE + Hg(II)] = 25 μM in H_2O.

Further solution studies on the stoichiometry of complexation were carried out. On the basis of the data obtained from the fluorescence titration experiments, where quenching proceeded rapidly up to the addition of 1.0 equiv of Hg(II) and was less influenced by the addition of molar excesses of Hg(II), we hypothesized that a 1:1 complex was likely formed between BnOQE and mercuric ion. This was confirmed using the method of continuous variation (Job's Method), where titrations were performed holding the concentration of Hg (II) and BnOQE constant while varying the mole ratios of both. A Job plot of the changes in fluorescence intensity produced at different ratios of sensor to Hg(II) showed a maximum quenching affect at a 0.5 mol ratio of BnOQE, which indicates a 1:1 solution stoichiometry for our aqueous system (Figure 5).

It remained to be seen whether or not collisional quenching caused by other ions would be a factor that limits the selectivity of the sensor for mercuric ion. Titrations were carried out as with Hg(II), where a 25 μM sample of BnOQE was treated with stock solutions of metal cations. As can be seen in Figure 6, the addition of molar excesses of Ag(I), Na(I), Ca(II), Cd(II), Cu(II), Fe(II), Mg(II), Mn(II), Pb(II), Zn(II) or Al(III) failed to completely turn

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[10] *Principles of Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.; Plenum Press: New York, 1983, p 257.

[11] Static quenching constants were obtained upon fitting of the curves to: $F_0/F = 1 + K_s[\mathrm{Hg(II)}]$

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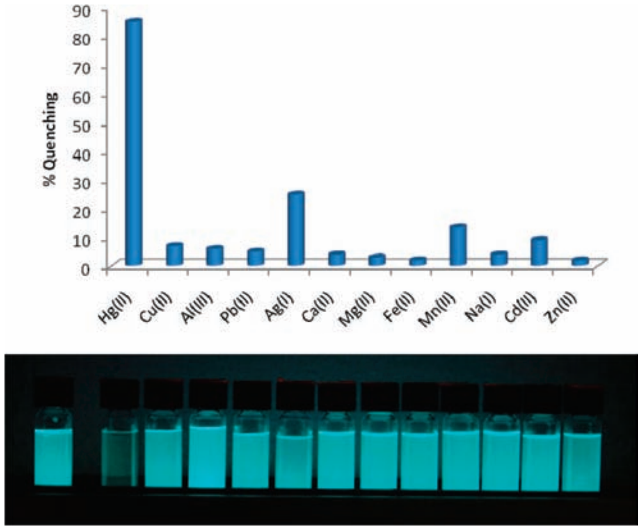

Figure 6. **(top)** Percentage of fluorescence quenched $(1-I/I_0)\%$ of BnOQE in the presence of 2 equiv of Hg(II), Cu(II), Al(III), Pb(II), Ag(I), Ca (II), Mg(II), Fe(II), Mn(II), Na(II), Cd(II) and Zn(II). [BnOQE] = 25 μM in H_2O, $\lambda_{ex} = 303$ nm. **(bottom)** Digital image of UV lamp (365 nm) visualization of (left to right): BnOQE, and BnOQE + 2 equiv each Hg(II), Cu(II), Al(III), Pb(II), Ag(I), Ca(II), Mg(II), Fe(II), Mn(II), Na(II), Cd (II) and Zn(II). [BnOQE] = 10^{-4} M in H_2O.

off the fluorescence of BnOQE. A series of samples prepared for visual analysis clearly showed this behavior (inset photo, Figure 6).

We noted that silver ion was the sole species tested that generated any significant (>20%) quenching of the fluorescence of BnOQE, indicating the need for a competition experiment to examine the selectivity of our sensor in the presence of a concomitant quencher.

Fortunately, when a water solution of BnOQE and 2 equiv of Ag(I) was titrated with Hg(II) (see Supporting Information), the fluorescence of the probe was quenched in the same fashion as when the titration was carried out with Hg(II) alone, demonstrating the selectivity of BnOQE for aqueous mercuric ion.

Conclusions

The facile synthesis of a structurally simple water-soluble 8-HQ-derived fluorophore exhibiting selective quenching in the presence of Hg(II) has been reported. To the best of our knowledge, this probe is the first mercuric ion-specific 8-HQ-derived ON-OFF fluorophore that functions in pure water. The probe allows for the determination of low μM concentrations (midppb and higher) of Hg(II). This makes the sensor particularly suitable for environmental applications where mercuric ion can be found in these concentrations in aqueous media. The ability of our probe to function in unbuffered solution may also lend to its use as a method for the rapid detection of Hg(II) in field settings.

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Supporting Information Available: Synthetic procedures and characterization data, copies of ^{1}H and ^{13}CNMR data for all compounds and Ag(I) competition titration data. This material is available free of charge via the Internet at http://pubs.acs.org.