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Optical Mercury Sensing Using a Benzothiazolium Hemicyanine Dye

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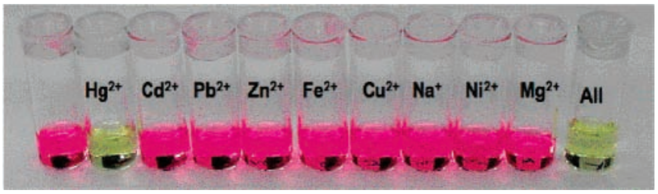
ABSTRACT



The selectivity and sensitivity of a benzothiazolium hemicyanine dye toward mercury(II) in aqueous solutions are described. Mercury ions coordinate to the dye forming a 1:1 complex. This interaction induces a color change in the dye at micromolar concentrations of mercury. Furthermore, the color change and quenching of the dye emission are selective for mercury when compared with other ions such as lead(II), cadmium(II), zinc(II), or iron(II).

Selective optical sensing is attracting strong interest due to the use of "low-tech" spectroscopic instrumentation to detect relevant chemical species in biological and environmental processes.[1,2] In particular, one aspect of environmental monitoring is the detection of low levels of heavy metals in aqueous solutions. Of these, mercury is of particular concern worldwide because of its high toxicity. Mercurial species may be released into the environment through coal-fired power plants and into water streams from chemical, gold mining, and chlor-alkali industrial plants.

Recently, several colorimetric probes, so-called "naked eye chemosensors", have been developed for the detection of small quantities of mercury.[2] Several groups have shown that molecular sensing based on ruthenium complexes is an attractive approach because of the sensitivity and mercury-selective properties of such complexes.[3] However, the use of such complexes might be seen as a demanding step toward the development of cheap sensing technology. For this reason, we have decided to move forward and synthesize an organic molecular probe (**OMP**) as mercury selective and colorimetrically responsive as ruthenium dyes but less expensive from a synthetic point of view. Furthermore, we have taken into account the possibility of using the **OMP** not only for colorimetric but also for fluorometric mercury sensing, increasing the sensitivity by using fluorescence emission spectroscopic methods.

On the basis of previous literature reports,[4] we decided to synthesize a molecular probe consisting of a charge-transfer complex whose electronic properties (UV−visible and

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(1) (a) Brümer, O.; La Clair, J. J.; Janda, K. D. *Org. Lett.* **1999**, *1*, 415−418. (b) Brümer, O.; La Clair, J. J.; Janda, K. D. *Bioorg. Med. Chem.* **2001**, *9*, 1067−1071.

(2) (a) Sancenón, F.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2001**, 2262−2263. (b) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127* 16760−16761. (c) Nolan, E. M.; Racine, M. E.; Lippard, S. J. *Inorg. Chem.* **2006**, *45*, 2742−2749. (d) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859−861. (e) Caballero, A.; Martínez, R.; Lloveras, V.; Ratera, I.; Vidal-Gancedo, J.; Wurst, K.; Tárraga, A.; Molina, P.; Veciana, J. *J. Am. Chem. Soc.* **2005**, *127*, 15666−15667.

(3) (a) Palomares, E.; Vilar, R.; Durrant, J. R. *Chem. Commun.* **2004**, 362−363. (b) Coronado, E.; Galan-Mascarós, J. R.; Marti-Gastaldo, C.; Palomares, E.; Durrant, J. R.; Vilar, R.; Gratzel, M.; Nazeruddin, Md. K. *J. Am. Chem. Soc.* **2005**, *127*, 12351−12356. (c) Nazeruddin, Md. K.; Di Censo, D.; Humphry-Baker, R.; Gratzel, M. *Adv. Func. Mater.* **2006**, *16*, 189−194.

(4) (a) Fedorova, O. A.; Fedorov, Y. V.; Vedernikov, A. I.; Gromov, S. P.; Yescheulova, O. V.; Alfimov, M. V.; Woerner, M.; Bossmann, S.; Braun, A.; Saltiel, J. *J. Phys. Chem. A* **2002**, *106*, 6213−6222. (b) Chen, Y.-S.; Li, C.; Zeng, Z.-H.; Wang, W.-B.; Wang, X.-S.; Zhang, B.-W. *J. Mater. Chem.* **2005**, *15*, 1654−1661. (c) Basheer, M. C.; Alex, S.; Thomas, G. K.; Suresh, H. Ch.; Das, S. *Tetrahedron* **2006**, *62*, 605−610.

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fluorescent emission spectra) can be strongly affected when mercury is present. Moreover, the molecular probe should contain mercury-coordinating elements to ensure high selectivity in the presence of other heavy metals. Hemicyanine dyes (Figure 1) provide such features. These molecules combine high molar extinction coefficients at the maximum absorption wavelength (in most cases $\epsilon \sim 10^4\ M^{-1}\ cm^{-1}$ in the visible region) with excellent emission quantum yields and good solubility in aqueous solutions.

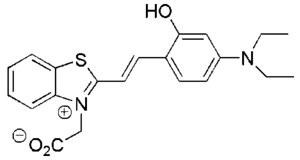

Figure 1. Molecular structure of **OMP**.

OMP is a hemicyanine dye composed of an electron-donor aniline moiety and an electron acceptor that in this case is a benzothiazolium species where the sulfur atom provides the mercury-coordinating element. This compound was easily synthesized in two steps from 2-methylbenzothiazole, bromoacetic acid, and 4-diethylamino-2-hydroxybenzaldehyde as a dark-green solid, in 54% overall yield.[4b]

Figure 2 shows the changes on the UV−visible spectra of **OMP** upon addition of mercury chloride at pH 7. As can be observed, the addition of mercury induces a clear isosbestic point corresponding to the presence of only two species at a constant total concentration of **OMP**.

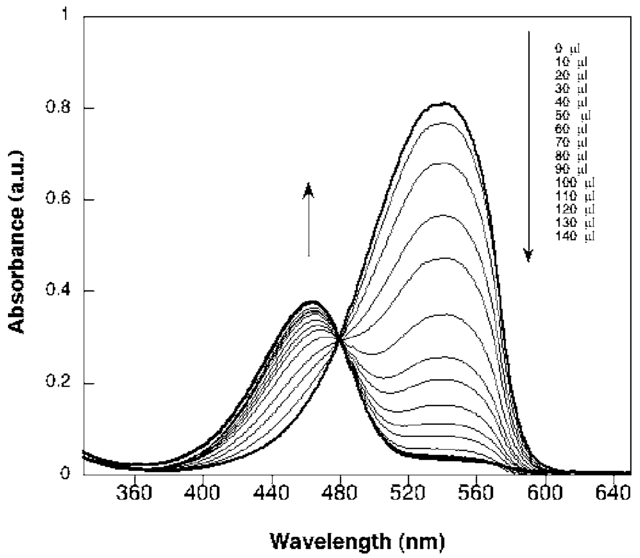

Figure 2. Changes in the UV−vis spectra of **OMP** (10 μM) in aqueous solution (ethanol/HEPES buffer, pH 7.0, v/v 1:10) upon addition of increasing amounts of $HgCl_2$ in water (1 mM). The total volume was 3 mL.

The results in Figure 2 are in good agreement with the Job's plot analysis (Figure 3), where the binding stoichiometry for the mercury/**OMP** complex was found to be 1:1 with a calculated association constant, K_a, of $1.0 \times 10^7\ M^{-1}$ in ethanol/HEPES buffer solution (1:10, v/v) at pH 7 (see Supporting Information), showing the high affinity of **OMP** for Hg^{2+}.

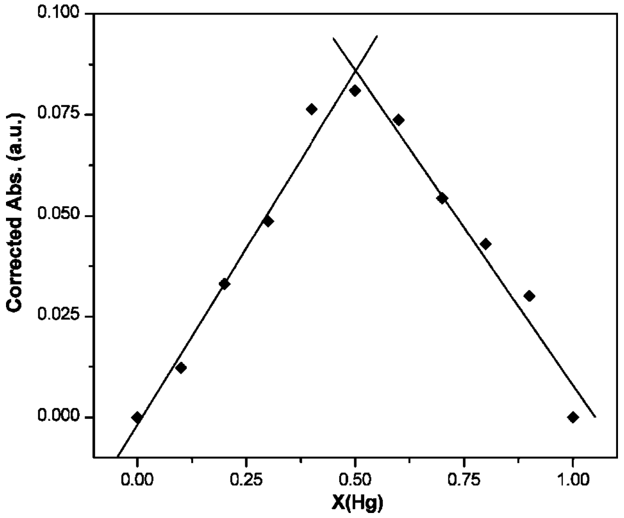

Figure 3. Job's plot of the complexation between **OMP** and Hg^{2+}. Total concentration of **OMP** + Hg^{2+} was kept constant at 10 mM in ethanol/HEPES buffer, pH 7.0, v/v 1:10.

In fact, the presence of mercury, at neutral pH, can be detected without the use of any instrumentation as illustrated in Figure 4. The selectivity toward mercury has been demonstrated even in the presence of similar amounts of other metal cations catalogued by the Environmental Protection Agency (EPA) as toxic substances. Moreover, the presence of common anions such as phosphate, chloride, iodide, and fluoride also did not change the electronic properties of **OMP**.

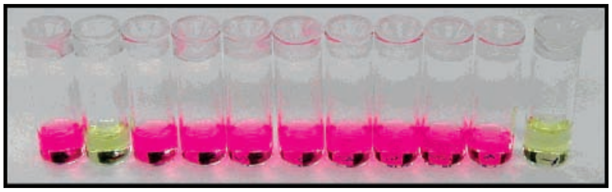

Figure 4. Digital picture of **OMP** in ethanol/HEPES buffer (pH 7) solution (v/v 1:10). From left to right: no metal, Hg^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Na^{+}, Ni^{2+}, Mg^{2+}, equivalent amounts of all the cations and Hg^{2+}. The cation total concentration was 0.1 mM.

Furthermore, to show the high mercury selectivity at low metal concentrations and to determine the sensitivity of **OMP**

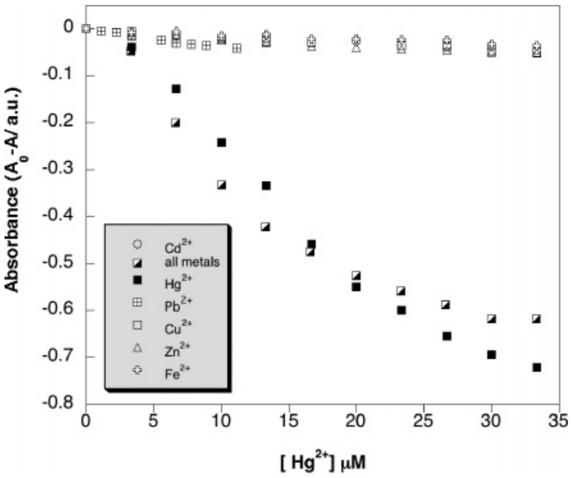

Figure 5. UV−visible titration of **OMP** in an aqueous solution (ethanol/HEPES buffer, pH 7 solution, v/v 1:10) in the presence of selected cations. The absorption wavelength was fixed at $\lambda = 538$ nm.

toward mercury, we used UV−visible spectroscopy. Figure 5 shows that in the presence of other metals the selectivity and sensitivity of our molecular probe **OMP** is barely affected.

Taking into account that most of the common metal cations could precipitate in basic pH conditions, we decided to perform some additional experiments using acidic and neutral pH controlled solutions. To evaluate the photophysical properties of the dye in acidic or basic media, we measured the UV−visible absorbance as a function of pH. Our experiments showed that the maximum of the UV−vis spectra was hardly affected in acidic and neutral conditions (Figure 6); however, in highly acidic media (pH < 3.5), the molar extinction coefficient for the organic molecule diminishes, and when a basic pH is used, the UV−visible spectra has a bathochromic shift of 25 nm. We expected this pH effect because of the nature of the charge-transfer band of the hemicyanine molecule, which depends on its protonation state.

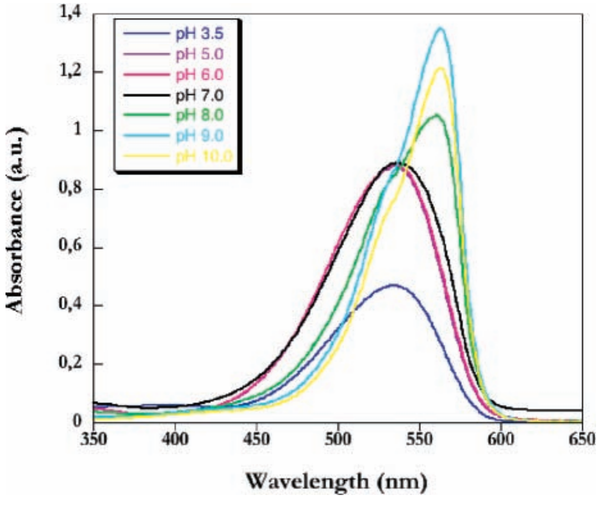

Figure 6. Absorbance of **OMP** at different pH values. The ligand total concentration was 0.1 mM.

As can be seen in Figure 7, the presence of mercury chloride induces changes on the absorbance of **OMP**. However, as expected, the response toward Hg^{2+} is different depending on the pH of the aqueous solution, obtaining the highest sensitivity at neutral pH.

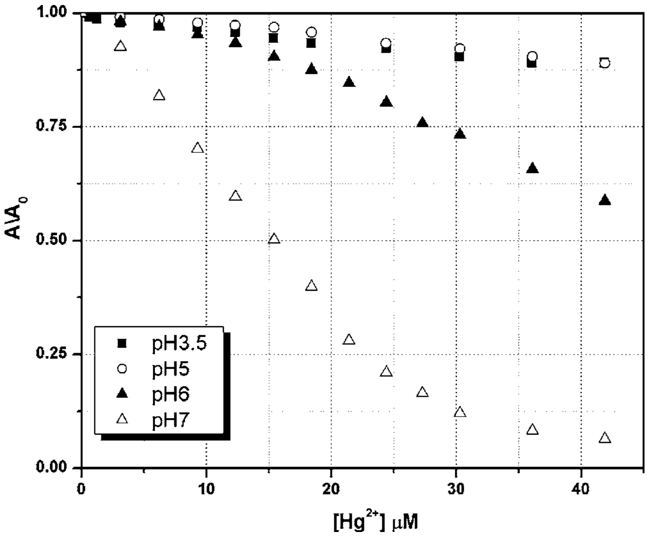

Figure 7. UV−vis response of **OMP** toward Hg^{2+} at different pH values registered at $\lambda = 535$ nm. The **OMP** concentration was 0.1 mM.

Turning to the fluorescent emission properties of **OMP**, it is well-known that fluorescent emission spectroscopy is more sensitive toward small changes that affect the electronic properties of molecular receptors. Several authors have based their chemosensors on this approach.[5] To check this method, we measured the fluorescent emission properties of **OMP** in the presence of mercury.

As illustrated in Figure 8, mercury has a strong quenching effect, where almost 85% of luminescence is quenched at room temperature. Moreover, the emission yield is barely affected by other toxic cations commonly used for mercury-interference experiments. Thus, **OMP** not only shows excellent colorimetric properties for mercury sensing but also can be used as a "turn-off" fluorometric molecular probe with high selectivity toward mercury and a detection limit of 100 ppb.[6]

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(5) (a) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270−14271. (b) Chen, Y.; Zeng, D. X. *Chem. Phys. Chem.* **2004**, *5*, 564−566. (c) Wang, J.; Qian, X. *Chem. Commun.* **2006**, 109−111. (d) Matsushita, M.; Meijer, M. M.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *Org. Lett.* **2005**, *7*, 4943−4946. (e) Zhang, H.; Han, L.-F.; Zacharlasse, K. A.; Jiang, Y.-B. *Org. Lett.* **2005**, *7*, 4217−4220. (f) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300−4302. (g) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968−969.

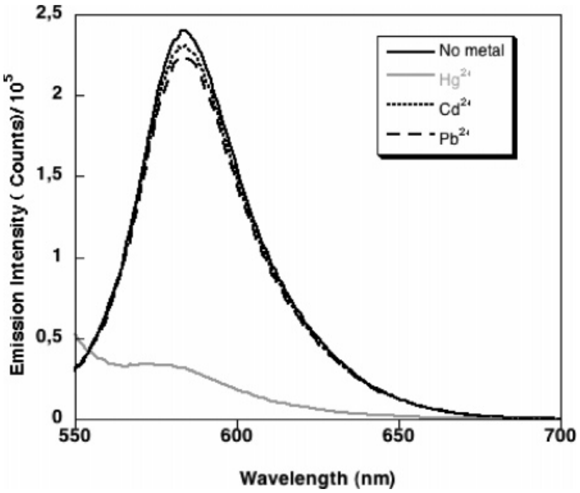

Figure 8. Fluorescent emission spectra under normal conditions of **OMP** in an aqueous solution (ethanol/HEPES buffer, pH 7 solution, v/v 1:10) (excitation wavelength $\lambda = 530$ nm). As can be seen, the emission is only strongly quenched by Hg^{2+} (~15 μM).

Finally, we would like to consider the chemical nature of the high selectivity of **OMP** toward mercury. ^1H NMR experiments of **OMP** in CD_3OD at different temperatures showed that the ligand is present as a mixture of *trans*- and *cis*-cyanines (***trans*-OMP** and ***cis*-OMP**) in dynamic equilibrium, with ***trans*-OMP** being the most stable isomer due to a minimum of steric repulsions. The planarity of the ***trans*-OMP** isomer optimizes the conjugation of the donor and acceptor moieties resulting in a strong coloration. In the presence of Hg^{2+}, **OMP** forms a relatively stable metal complex with a 1:1 stoichiometry, involving the sulfur atom of the benzothiazolium moiety and most probably also the oxygen atom of the phenolic group, forcing the hemicyanine to adopt a cis configuration. In the ***cis*-OMP** isomer, both chromophores are nonplanar, inducing a hypsochromic shift on the UV−visible spectrum. Other groups have reported a similar stabilization of a related *cis*-merocyanine isomer via intramolecular chelation to transition metals.[4a,7] This hypothesis is supported by the fact that in a highly acidic media the sensitivity of **OMP** toward mercury decreases. Most probably, the nitrogen atom of the donor moiety will be protonated, inhibiting the thermal trans/cis isomerization of the central double bond.

In conclusion we have demonstrated selective colorimetric and fluorometric mercury sensing using a benzothiazolium hemicyanine dye in aqueous solution with a response limit in the case of fluorescence spectroscopy of 100 ppb. The high selective nature of the molecular probe toward mercury is probably due to the presence of the sulfur atom and to the fact that mercury ions induce a geometric change from the trans isomer to the cis form, as can be deduced from the results of simple spectroscopic measurements. Further efforts toward understanding and controlling the mercury uptake by light switching between both dye stereoisomers are currently in progress. We see these initial results as the first step toward light-induced molecular sensors in the presence of mercury ions.

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Supporting Information Available: Experimental details and characterization data for **OMP** and mercury binding constant determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) The response limit was calculated as the minimum concentration that can be determined with 99% of confidence that the true concentration is greater than zero. See: Guidelines establishing test procedures for the analysis of pollutants. Appendix B: Definition and Procedure of the Determination of the Method Detection Limit (MDL). http://ecfr.gpoaccess.gov.

(7) Wojtyk, J. T. C.; Kazmaier, P. M.; Buncel, E. *Chem. Mater*. **2001**, *13*, 2547−2551.