Analytical Chemistry

A Regenerative Chemodosimeter Based on Metal-Induced Dye Formation for the Highly Selective and Sensitive Optical Determination of Hg^{2+} Ions**

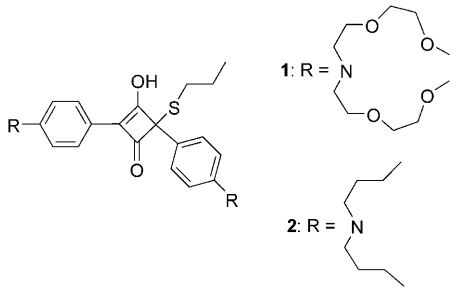
José V. Ros-Lis, M. Dolores Marcos, Ramón Mártinez-Máñez, Knut Rurack,* and Juan Soto*

The Hg^{2+} ion is one of the environmentally most important metal ions whose toxicity, even at very low concentrations, has long been recognized and is a problem of primary concern.[1] Moreover, despite a reduction of its industrial use as a result of stricter regulations, high concentrations of mercury are still present in many environmental compartments and it can still be found in many products of daily life such as paints, electronic equipment, and batteries.[2] Accordingly, the need for analytical methods for the sensitive and selective determination of mercury is of topical interest, especially in situations where conventional techniques are not appropriate, for instance in many on-site or in situ analyses and for rapid screening applications. In these fields, optical and electrochemical sensing devices play a leading role, in particular utilizing molecular probes that generate and transduce an analytical signal as a response to the binding event.[3] Although several examples of redox-active,[4] fluorogenic,[5] and chromogenic[6] chemosensors for Hg^{2+} ions have been reported recently, the combined "binding site and signaling subunit" approach that is commonly used with such probes often harbors disadvantages for analysis in realistic media. Many signaling units suffer through the strong hydrogen-bonding ability of water, fluoroionophores often undergo unspecific fluorescence quenching upon binding to heavy-metal ions, and the number of Hg^{2+}-ion-selective receptors is limited.

A particularly attractive alternative presented herein are chemodosimeters that indicate an analyte through a specific chemical reaction between dosimeter molecule and target species, leading to the formation of a fluorescent or colored product.[7,8] A disadvantage of dosimeters, however, is that the reactions are mostly irreversible and thus provide only single-use assays.

To take advantage of the favorable features of chemodosimeters and also reuse them, it is necessary to install a procedure that allows the molecular reporter to be regenerated. We thus designed a method where the indicator dye is passivated first in a chemical addition reaction with a small organic compound, the "spectroscopic inhibitor" that "switches off" the color and fluorescence of the indicator. This addition product is the chemodosimeter. The target ion then reacts with the inhibitor, liberating the dye, that is, signaling is accomplished by "metal-induced dye release" methods. With the proper choice of dye scaffold and passivation reaction, it should thus be possible to generate drastic chromo- and fluorogenic changes that result in a true "switching-on" behavior rather than a modulation of already existing signals or minor shifts of absorption or emission bands.

We have applied this newly designed procedure to the selective analysis of Hg^{2+} ions. The method is based on the thiophilic affinity of the Hg^{2+} ion and its reactivity with 2,4-bis(4-dialkylaminophenyl)-3-hydroxy-4-alkylsulfanylcyclobut-2-enone (APC) derivatives. As we have recently shown, APC derivatives can be elegantly obtained by a simple reaction of squaraines—the indicator dye—with thiols, the inhibitor.[9] A representative compound (**1**) is shown in Scheme 1. Compound **1** contains two independent subchromophores that are electronically separated and chemically bound through an sp^3-hybridized carbon atom. Accordingly, **1** shows two overlapping bands in the UV spectral region; one at approximately 265 nm which is characteristic for the dialkylanilino moiety and a second, 1.3-fold more intensive band centered at around 305 nm (Figure 1). The latter band can be ascribed to the dialkylaminophenylhydroxycyclobut-2-enone (PC) group. Quantum chemical calculations at the semiempirical level are in agreement with the experimental results and reveal the existence of two independent transitions of moderate oscillator strength, the HOMO–LUMO transition that is located on the PC fragment and the energetically higher-lying HOMO-1–LUMO + 1 transition localized on the anilino group.



Scheme 1. Derivatives **1** and **2**.

Addition of Hg^{2+} ions to solutions of **1** results in a dramatic change of color owing to the appearance of a new

[*] J. V. Ros-Lis, Dr. M. D. Marcos, Prof. R. Mártinez-Máñez, Dr. J. Soto
Centro de Investigación en Química Molecular Aplicada
Departamento de Química
Universidad Politécnica de Valencia
Camino de Vera s/n, 46071 Valencia (Spain)
Fax: (+34) 96-387-9349
E-mail: rmaez@qim.upv.es
Dr. K. Rurack
Div. I.3
Bundesanstalt für Materialforschung und -prüfung (BAM)
Richard-Willstätter-Strasse 11, 12489 Berlin (Germany)
Fax: (+49) 30-8104-5005
E-mail: knut.rurack@bam.de

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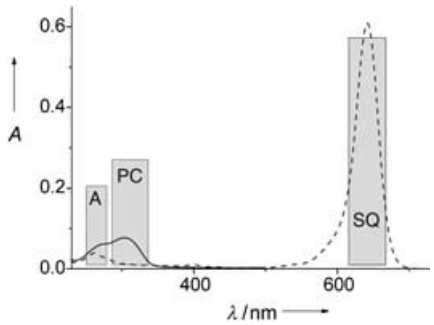
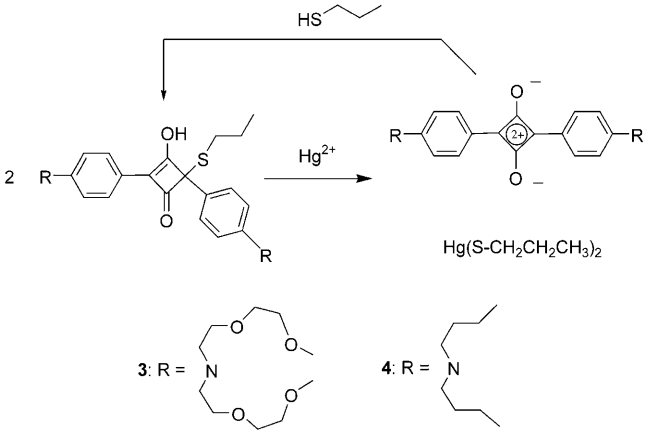


Figure 1. Absorption spectra of **1** (——) and **3** (-----) in water/acetonitrile (4:1 v/v), before and after the reaction of **1** with Hg^{2+} ions. The gray bars denote the region of absorption of the single chromophores: A = anilino moiety (this band is also present as a higher transition in **3**), PC = aminophenylhydroxycyclobut-2-enone group and SQ = squaraine.

and intense absorption band at 642 nm that is typical for a squaraine dye (Figure 1).[9,10] This Hg^{2+}-ion triggered formation of squaraines is shown in Scheme 2. The reaction product



Scheme 2. The analytical cycle: Hg^{2+}-ion-induced formation of **3** and **4** and regeneration of **1** and **2** by reaction with propanethiol.

3 has a comparatively large molar absorptivity ($\lg\varepsilon = 5.05$) and a moderately high fluorescence quantum yield ($\Phi_f = 0.023$) in water/acetonitrile (4:1). This dual chromo- and fluorogenic sensing of Hg^{2+} ions is remarkable as both absorption and emission are found at the red end of the visible spectral window where usually the interference arising from matrix absorption or autofluorescence is negligible. Moreover, this dramatic hyper- and bathochromic shift upon mercury-induced transformation of **1** into **3** allows for straightforward "naked-eye" monitoring of the Hg^{2+}-ion-detection process.[11]

To optimize the method, different pH ranges were tested and a basic pH (9.6, buffered with 2-(cyclohexylamino)ethanesulfonic acid (CHES) 0.01M) was found to yield the best performance of the chromofluorogenic dosimeter. Furthermore, these studies revealed that a maximum signal can be observed at a molar ratio of **1**/Hg^{2+} = 2:1, suggesting the formation of $Hg(SR)_2$ species (see Scheme 2).

As the coordination with thiols is not an exclusive feature of mercury, the reactivity of **1** in the presence of other metal ions was studied. In a typical experiment, up to 10 equivalents of Hg^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Al^{3+}, and Tl^{+} were added to a solution of **1** in water/acetonitrile (4:1, $c_1 = 6\times10^{-6}$ M, pH 9.6, CHES 0.01M). The results are shown in Figure 2. There is a remarkably selective development of

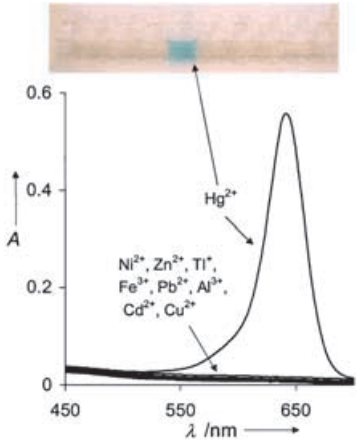


Figure 2. Absorption spectra of **1** (6×10^{-6} M) in water/acetonitrile (4:1 v/v, pH 9.6, CHES 0.01 M) upon addition of 0.5 equivalents of various metal ions. In the photograph, from left to right: Ni^{2+}, Zn^{2+}, Tl^{+}, Fe^{3+}, Hg^{2+}, Pb^{2+}, Al^{3+}, Cd^{2+}, Cu^{2+}, and no metal ion.

the blue color only in the presence of Hg^{2+}, while the other potential competitors remain entirely silent. This chromogenic indication reaction allows Hg^{2+} ions to be detected down to 20 ppb by using a conventional spectrophotometer and standard conditions (such as 10-mm cells).

Despite the inherent interest of the colorimetric response, fluorometric methods can reach much lower detection limits with very low-cost and widely used instrumentation. Considering the high brightness (the product of ε and Φ_f) of squaraine dyes, the present system should be ideal for use in conjunction with fluorescence detection. Thus, similar studies to those described above were performed with $c_1 = 1\times10^{-7}$ M on a conventional fluorometer. Figure 3 shows the fluorescence titration spectra of **1** with Hg^{2+} ions, revealing that the

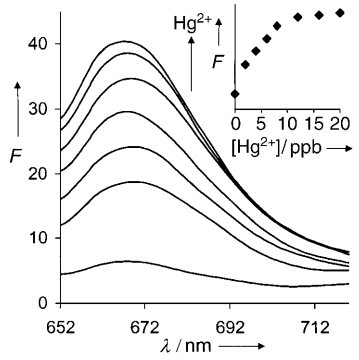


Figure 3. Fluorescence titration spectra of **1** (10^{-7} M) with Hg^{2+} ions in water/acetonitrile (4:1 v/v, pH 9.6, CHES 0.01 M, $\lambda_{exc} = 642$ nm). The inset shows the titration curve obtained from the emission intensities at 670 nm.

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detection of less than 2 ppb of Hg^{2+}, which is the U.S. Environmental Protection Agency's limit for drinking water, is clearly possible by the Hg^{2+}-ion-induced release of the highly fluorescent squaraine **3**.

Motivated by these favorable features of the solution-based chemistry, we took a step towards the use of metal-triggered reactions for in situ sensing and rapid screening applications. For this purpose, the APC derivative **2** (Scheme 1) containing hydrophobic *n*-butyl chains was prepared and adsorbed on powdered silica. The butyl chains offer the required hydrophobicity to prevent the leaching of the dye in aqueous samples. This rather simple method allowed an accurate control of the amount of dye that is physisorbed onto the solid. The loading is a key parameter for fluorometric applications as too high loadings can easily lead to self-quenching for dyes with narrow, intense, and weakly Stokes-shifted absorption and fluorescence spectra.

In a series of experiments we found that a suitably sensitive solid able to show dual chromofluorogenic signaling should typically contain **2** in a concentration of 10^{-6} mmol g^{-1}. The powder was then fixed on a polyethyleneterephthalate film for a first prototype of a dip-stick assay that was tested by simply dipping the films into aqueous samples containing Hg^{2+} ions. Once the sticks come into contact with the sample solutions, the solid turns blue ($\lambda_{abs} = 642$ nm) and begins to fluoresce ($\lambda_{em} = 670$ nm). The response time is on the order of a few seconds and the behavior is consistent with the reaction shown in Scheme 2. Compound **2** reacts with the mercuric ion to give the corresponding squaraine derivative **4** that remains adsorbed on the solid, resulting in a chromo- and fluorogenic "switching-on" process indicative of the presence of Hg^{2+} ions. Finally, having accomplished the reaction, the chemical reaction described above[9] can be used to regenerate the chemodosimetrical solid quantitatively with propanethiol (Scheme 2). Upon addition of the "spectroscopic inhibitor", the conjugation in the squaraine is interrupted and the color immediately turns from blue back to colorless; the device is ready for the next cycle (see the Supporting Information).

In summary, we have designed a highly selective and sensitive dual chromofluorogenic chemodosimeter system for the determination of Hg^{2+} ions in aqueous environments using a very simple and specific reaction, that is, Hg^{2+}-triggered formation of a squaraine dye. The method allows the selective detection of Hg^{2+} ions below 2 ppb in aqueous solutions, shows a fluorescence enhancement, and displays a very large shift in absorption with the development of a blue color from colorless solutions. The possibility to adsorb or anchor the probe on suitable supports allowed prototypes of reusable dip-stick assays to be designed for rapid screening for the target analyte. Additionally, we believe that this procedure, based on guest-induced dye release methods using suitable dye scaffoldings and passivation reactions, might be of interest as a new route for the design of new and improved regenerative sensor molecules for the rapid colorimetric screening of a number of other target guests.

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