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Colorimetric Hg^{2+} Sensing in Water: From Molecules toward Low-Cost Solid Devices

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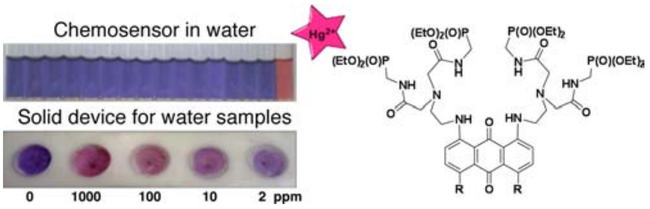
ABSTRACT



A new colorimetric molecular sensor allowing for cheap, fast, sensitive, and highly selective naked-eye detection of Hg^{2+} in water is described. This molecule combines a 1,8-diaminoanthraquinone signaling subunit and phosphonic acid esters that confer the water solubility to the dye (R = H). A ready-to-use colorimetric solid sensor was obtained by incorporating an amphiphilic analog (R = $OC_{12}H_{25}$) exhibiting similar binding properties and optical responses in an agarose film.

Toxic d^{10} cations such as Hg^{2+}, Cd^{2+}, and Pb^{2+} are widely distributed in the environment as a result of natural processes and anthropogenic activities. These elements constitute a very important group of contaminants since they are potent metabolic poisons for plants, animals, and humans. Unquestionably, the development of facile and efficient methodologies for monitoring these metal ions in a wide range of environmental samples or living systems is a continuing endeavor that has attracted much attention, although some breakthroughs are still anticipated.[1] Highly specific, water-soluble colorimetric detectors that respond by a sharp color change to the presence of Hg^{2+} are sought for fast and reliable visual detection or spectrophotometric quantification at the ppm to ppb concentration level.[2]

In this regard the rational design of effective chemosensors is of paramount importance to achieve high affinity and selectivity together with the fast binding kinetics of the coordination pocket, and high sensitivity of the signaling unit.[3] Ideally, operational molecular detectors should fulfill additional criteria, such as water solubility, insensitivity to pH changes, nontoxicity, and the widest possible range of linear response to the targeted cation concentrations. Besides, colorimetric sensors have also found widespread analytical applications once immobilized on dipstick sensors or incorporated in films or membranes. In that respect, the ability to tune the lipophilicity without affecting the sensing properties is another issue to be considered. Moreover, innovative rational synthetic approaches should be explored in preparing more efficient chemosensors which do not suffer from practical limitations such as

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low solubility in aqueous media or interferences by other environmentally important cations.

Pd- and Cu-catalyzed C−N cross-coupling reactions of aryl halides with amines enable direct conjugation of the receptor and signaling units, without introducing an additional spacer.[4] The direct connection of the aromatic signaling unit with the binding pocket is highly suited for an efficient signal transduction. This reaction can be successfully applied to the synthesis of optical chemosensors due to mild experimental conditions and its high tolerance to the chemical nature of substrates.[5] Accordingly, we recently reported the synthesis of two colorimetric chemosensors that exhibited high sensitivity and selectivity for Pb^{2+} and Cu^{2+} in aqueous media.[6] Herein, we describe a water-soluble and highly specific colorimetric Hg^{2+} indicator (**1**) and its amphiphilic structural analog (**2**), which incorporate one anthraquinone,[7,8] carbamoyls, amines, and phosphoester biocompatible groups (Figure 1). The latter is of particular interest for the development of sensing devices and can be used as a molecular precursor for different immobilization procedures including the Langmuir−Blodgett technique.

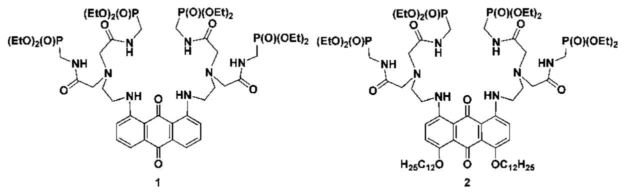
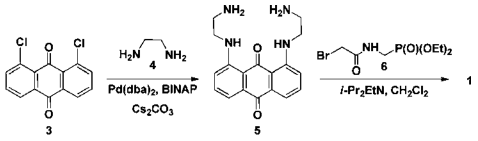


Figure 1. Structure of the Hg^{2+} chemosensors **1** and **2** based on the 1,8-diaminoanthraquinone chromophore.

The synthetic approach leading to receptor **1** is summarized in Scheme 1. The parent tetraamine **5** was synthesized from 1,8-dichloroanthraquinone (**3**) and diaminoethane (**4**) according to the Buchwald−Hartwig amination reaction in the presence of $Pd(dba)_2$/BINAP precatalyst and Cs_2CO_3 as a base.[9] **5** was obtained in 41% yield after purification by column chromatography on silica gel. In the final step, *N*-functionalization of **5** was achieved by alkylation using [(2-bromoacetylamino)methyl]phosphonic acid diethyl ester (**6**), prepared according to a literature procedure.[10] The best yield of receptor **1** was obtained when the reaction was carried out in refluxing CH_2Cl_2 using *i*-Pr_2EtN as a base. The product was obtained after purification by column chromatography with a yield close to 50%.

Scheme 1. Synthesis of Receptor **1**



The water-soluble compound **1** strongly absorbs in the visible region due to an intramolecular charge transfer (ICT) band (λ_{max} = 561 nm, ε = 9475 M^{-1} cm^{-1}) responsible for its blue color. Since protonation of the tertiary amines might compete with metal binding, the acid−base properties of **1** were evaluated by UV−vis spectrophotometry. As no spectral change occurs in the pH range 3−12, it can be concluded that **1** remains unprotonated above pH 3 where it can be used for detection purposes without any buffer (Figures S1−S2, Supporting Information (SI)). Below pH 3, hypsochromic shifts of the ICT band together with a slight color change evidence the very weak basicity of the tertiary amines of **1**, which is assigned to the strong electron-withdrawing character of the appended carbamoyl arms.[6b]

The metal-ion sensing properties of receptor **1** (0.13 mM) were first assessed visually and then by UV−vis spectrophotometry in deionized water (pH ≈ 5.6) by adding 10 equiv of 14 environmentally relevant cations (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+}, Al^{3+}). Among them, Hg^{2+} was the only one that produced an instantaneous color change from blue to pink (Figure 2a), as a consequence of the blue shift of the ICT absorption band (λ_{max} = 546 nm for 1 equiv) that reaches its maximum extent (λ_{max} = 509 nm) for 5 equiv (Figure 2b). The visual detection limit (DL) is estimated to be ~4 ppm (20 μM) from a cascade dilution experiment of a starting solution containing chemosensor **1** (0.13 mM) and 5 equiv of $Hg(ClO_4)_2$. By using a double-beam laboratory spectrophotometer in conjunction with a 1 cm quartz cell, the DL is decreased to 60 ppb (0.3 μM) at 509 nm. The fluorimetric detection has also been considered, but the emission intensity change of receptor **1** in the presence of Hg^{2+} was too low to significantly decrease the DL found by colorimetry (SI). These results entitle ligand **1** as one of the very first fully water-soluble chemosensors reported so far,

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allowing direct naked-eye mercury detection without additional cosolvent or surfactant.[2b]

Further spectrophotometric measurements (Figures S3−S12, SI) confirmed the lack of affinity of **1** (0.13 mM) for not only Pb^{2+} up to 10 equiv but also Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^+, and Al^{3+} even in the presence of a 100-fold excess of the corresponding perchlorate salt. In turn, Cu^{2+} and Cd^{2+} induce a slight but significant hypochromic effect on the ICT band of **1**, although complexation of these ions remains imperceptible to the naked eye. This is mainly due to the much weaker binding affinity compared to Hg^{2+}, which is supported by spectral changes still occurring beyond 5 equiv of Cu^{2+} and Cd^{2+}. Moreover, Cd^{2+} uptake does not involve any significant red or blue band shift, thus producing no color change. Receptor **1** can effectively discriminate Hg^{2+} from other cations in aqueous media. Most importantly, none of the above-mentioned 13 ions impair the colorimetric detection of Hg^{2+} as could be deduced from interference studies. There was no marked change in color and absorption properties when different cations (10 equiv for transition metals or 100 equiv for alkaline and earth-alkaline ions) were added to a 0.13 mM solution of **1** containing 5 equiv of Hg^{2+} and vice versa (Figures S13−S21, SI).

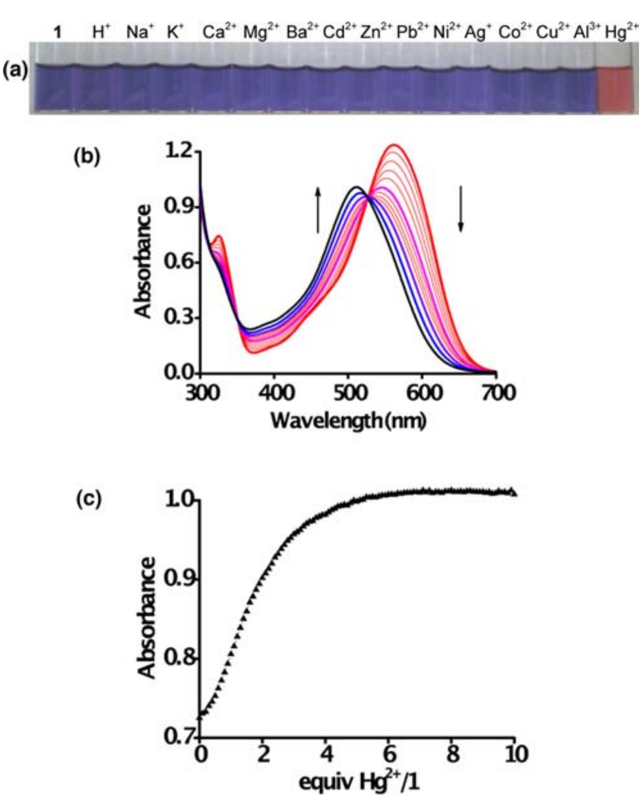

Figure 2. (a) Cation-induced color changes of chemosensor **1** (0.13 mM) in aqueous solution upon addition of 10 equiv of metal salts. (b) Evolution of UV−vis spectrum of **1** upon addition of 0 (bold red), 1 (magenta), 2 (violet), 3 (blue), and 5 equiv (black) of Hg^{2+} (full data are displayed in Figure S22 (SI)). (c) Changes of absorption intensity at 509 nm upon addition of Hg^{2+}. Spectrophotometric titration of the Hg^{2+}/**1** system (5:1 concentration ratio) as a function of pH is shown in Figure S25 (SI).

To gain a deeper insight into the mercury binding properties of **1** in water, spectrophotometric titrations of a 0.13 mM solution of chemosensor **1** were performed by adding a $Hg(ClO_4)_2$ solution (23 mM in CH_3CN) in 1 μL aliquots. As seen in Figures 2b and S22 (SI), the gradual hypsochromic shift of the ICT absorption band maximum goes together with a sharp intensity decrease up to the addition of 2 equiv of Hg^{2+}, followed by an increase until final stabilization is reached for 5 equiv (Figure 2c). The lack of isosbestic points suggests the formation of at least two distinct absorbing complexes in equilibrium with the free ligand. Numerical data processing with the Specfit program[11] showed that the best fit corresponded to a model including the mono- and dinuclear $[Hg(\mathbf{1})]^{2+}$ (log K_{11} = 5.31(2)) and $[Hg_2(\mathbf{1})]^{4+}$ (log K_{21} = 4.02(6)) complexes. The latter are fully dissociated at pH 1−2, as pure ligand can be recovered upon acidification and extraction into chloroform. Distribution diagrams and calculated electronic absorption spectra are displayed in the SI (Figures S23−S24). For both complexes, involvement of the aminoanthraquinoyl moiety in the Hg^{2+} binding scheme is strongly supported by the blue-shifted ICT band with respect to the free ligand.[6b]

High-resolution ESI-MS measurements in methanol/water mixtures (SI) confirmed the $[Hg(\mathbf{1})]^{2+}$ and $[Hg_2(\mathbf{1})]^{4+}$ stoichiometries deduced from UV−vis titrations. Structural information on these species was tentatively gained by ^1H and ^{31}P NMR spectroscopy in D_2O. Despite some poorly resolved ^1H NMR spectra, pronounced spectral changes were observed in both low and high field regions upon titrating chemosensor **1** with Hg^{2+}, reflecting stepwise coordination. Noteworthy, the ^1H and ^{31}P NMR signals assigned to the diethoxyphosphoryl groups are markedly shifted from the beginning of the titration, suggesting their involvement in Hg^{2+} binding. The most valuable structural information could be obtained from the partially resolved ^1H NMR spectrum recorded in the presence of 4 equiv of salt, under which conditions $[Hg_2(\mathbf{1})]^{4+}$ is the predominating solution species. The number of distinct proton signals does not change after Hg^{2+} ion complexation, indicating a time-averaged C_{2v} symmetry of the solution structure of the complex. Based on symmetry arguments and the large complexation-induced shifts exhibited by all protons, it can be assumed that the tertiary amines, one anthraquinone O-atom, and the carbamoyl and phosphoryl groups are involved in the coordination, in such a way that each polyamine branch binds a single metal ion. However, in the absence of a crystal structure, the exact binding mode of the amide moieties (Hg−O or Hg−N coordination) remains unclear. The binding of carbamoyl and phosphoryl O-atoms to the metal center would result in 7-membered chelate cycles, the latter being considered as unstable.[12]

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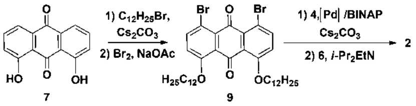
Conversely, ligation of the deprotonated amidic N-atoms, as described for Hg^{2+} complexes formed with cyclic amides,[13] would result in stable 5-membered cycles.

Prompted by the successful selective detection of Hg^{2+} in homogeneous aqueous media, an amphiphilic analog of receptor **1** was prepared with the aim of developing solid sensor arrays such as multiwell plates, dip-sticks, or paper strips. Moreover, amphiphilic chemosensors incorporated in a monolayer at the air/water interface or in films deposited on a solid surface according to the Langmuir–Blodgett technology represent a new and promising paradigm in metal detection. To that end, the 4,5-disubstituted analog **2** bearing dodecyl chains was obtained in four steps (Scheme 2), starting from commercially available 1,8-dihydroxyanthraquinone (**7**). Alkylation followed by bromination afforded the 4,5-dialkoxy-1,8-dibromoanthraquinone **9** that was subjected to Buchwald–Hartwig amination with diamino ethane (**4**) followed by *N*-substitution with the bromo derivative **6**.

Scheme 2. Synthesis of Chemosensor **2**



Compound **2** exhibits good water solubility in the 0.1 mM concentration range and sensing properties equivalent to those described for the parent indicator **1**. Likewise, spectrophotometric titrations conducted in pure water evidenced a 30 nm blue shift of the absorption maximum (λ_{max} = 565 nm, ε = 10350 M^{-1} cm^{-1}) of **2** upon addition of 5 equiv of Hg^{2+}, which accounts for the blue-violet to pink color change of the solution. Thus, appendage of both alkyl chains to the anthraquinone ring impacts neither the optical nor the thermodynamic properties, as virtually identical apparent stepwise formation constants were refined for the mono- and binuclear complexes of **2** (log K_{11} = 5.4(2) and log K_{21} = 4.0(2)).

Thanks to its high affinity and selectivity for Hg^{2+}, chemosensor **2** is a promising molecular precursor for manufacturing thin-film sensing systems. En route to the development of solid-state detectors, air-dried disks of polymer films (agarose, Type I-B) impregnated with 0.5% of **2** were cut out and deposited in the loculi of a Teflon plate. For that purpose, dye **2** appeared to be better suited than **1** due to the higher stability of the film when it came into contact with the aqueous test solutions (10 μL). As shown in Figure 3, this simple and convenient probe enables the fast and steady-going semiquantitative determination of Hg^{2+} ions at the ppm level. Although the time required to observe the definitive color was concentration dependent, an immediate change was obtained in the 100–1000 ppm range, while the response time for detecting 2 ppm of Hg^{2+} was 3 min.

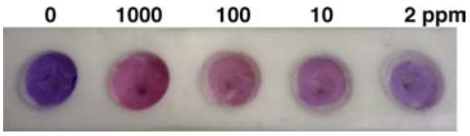


Figure 3. Visual detection of 2–1000 ppm (0.01–5 mM) of Hg^{2+} in aqueous solutions using small disks of an agarose polymer film doped with chemosensor **2** at 0.5% w/w.

In conclusion, it is demonstrated for the first time that water-soluble anthraquinone-based chemosensors **1** and **2** endowed with hydrophilic diethoxyphosphoryl binding groups allow for cheap, fast, sensitive, and highly selective detection of Hg^{2+} in aqueous solutions. Thanks to their very weak basicity and high absorptivity in the visible region, dye **1** and its amphiphilic counterpart **2** are suitable reagents for direct colorimetric determination of Hg^{2+} in water over a wide concentration range, without the need to buffer the medium. As a first step toward the development of advanced solid sensors that enable the rapid and reliable on-field detection of toxic metals, a simple dip probe was devised. Semiquantitative recognition of Hg^{2+} at the ppm level could be achieved with a polymer film doped with chemosensor **2** placed in the loculi of a Teflon plate. Binding studies of monolayers transferred on solid substrates using the Langmuir–Blodgett methodology are currently underway and will be reported in due course. Once miniaturized, these 2D film deposits can be considered as a "lab on the chip" paradigm for the molecular-based Hg^{2+} recognition.

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Supporting Information Available. Experimental section, UV–vis absorption spectra, spectrophotometric titrations, estimation of the detection limit, MS, ^{1}H, ^{13}C, and ^{31}P NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.