Analytical Microsystems

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Towards an Efficient Microsystem for the Real-Time Detection and Quantification of Mercury in Water Based on a Specifically Designed Fluorogenic Binary Task-Specific Ionic Liquid**

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The control of water resources and the natural environment is a major current societal challenge that involves various partners at different levels (local government, water agencies, water treatment stations, citizens, consumer protection organizations, etc.). In this general context, facing the ecological hazards originating from the presence of heavy-metal ions released in the environment (including water resources) is one of the important issues to address. Heavy-metal ions such as mercury cause adverse environmental and health problems such as bioaccumulation by numerous organisms and severe physiological problems, including neurological, neuromuscular, or nephritic disorders. Indeed, mercury is considered the most toxic nonradioactive metal. Among the different forms (including the toxic organic derivative methylmercury), mercuric ions (Hg^{2+}) are not only toxic but also highly water-soluble, making them bioavailable for humans and animals by ingestion of water. The toxicity associated with the bioavailability of Hg^{2+} ions entails an increasing number of analyses to be carried out both on tap water and on water resources. Although laboratory analyses such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption or atomic emission spectroscopy are appropriate for precise quantitative measurements, they suffer from several drawbacks, including high cost and duration. Moreover the "in-lab" techniques cannot be readily used for real-time in situ analysis, which is required to monitor transient events such as release from thunderstorm overflow. Alternative selective Hg^{2+} sensors have been reported based upon optical,[1] electrochemical,[2] electrical,[3] or biological detection methods.[4] These sensors allow in situ mercury monitoring, thus avoiding the use of laboratory analyses. However, most sensors are limited with respect to sensitivity[5] or are not adapted for real-time monitoring of transient phenomena requiring high measurement repetition rates.

This situation clearly calls for the development of efficient alternatives for in situ sensitive monitoring of water quality, which is subject to several official norms.[6] To overcome this limitation, we herein propose a high-performance micro-total analysis system (μTAS), which is based on both the use of a novel selective and sensitive molecular fluorescent sensor in an ionic liquid phase and on liquid–liquid extraction, thus allowing concentrating extraction of the heavy-metal cation from a water phase. Several authors[7] have reported the extraction of heavy metals with ionic liquids containing crown ethers with very high Nernst coefficients. Furthermore, the nonvolatility of ionic liquids enables their use in very low volume in open systems.[8] One of the most recent developments in the field of ionic liquids is the use of task-specific ionic liquids (TSILs) or task-specific onium salts (TSOSs) as soluble supports, therefore expanding their potential applications far beyond those of conventional ionic liquids. TSILs retain all of the advantages of ionic liquids and can be used for liquid–liquid extraction[9] with important benefits. Furthermore, TSILs (or TSOSs) can be dissolved in room-temperature ILs to give binary solutions combining all of the above-mentioned benefits.

Liquid–liquid extraction microsystems[10] have been intensively developed during the last 10 years to extract, concentrate, and detect target molecules present in low concentrations in a liquid. Various solutions have been proposed to stabilize the interfaces, such as coflowing immiscible liquids separated by microridges, microporous membranes, or micropillars. However, classical organic solvents with poor Nernst coefficients were used in those microsystems, thus limiting their efficiency. In contrast, ionic liquids provide unique remarkable extraction abilities owing to their very high Nernst coefficient[9] for metal-ion extraction. On the basis of the above analysis, we herein report the implementation and investigation of a novel and efficient microdevice for liquid–liquid extraction and sensing of mercury ions in water. The concept is based on an ionic liquid containing a TSOS, which combines fluorogenic and chelating properties to yield fluorescent-sensor ionic liquids (FSILs). Such immobilization of the metal binding unit in a hydrophobic IL would greatly

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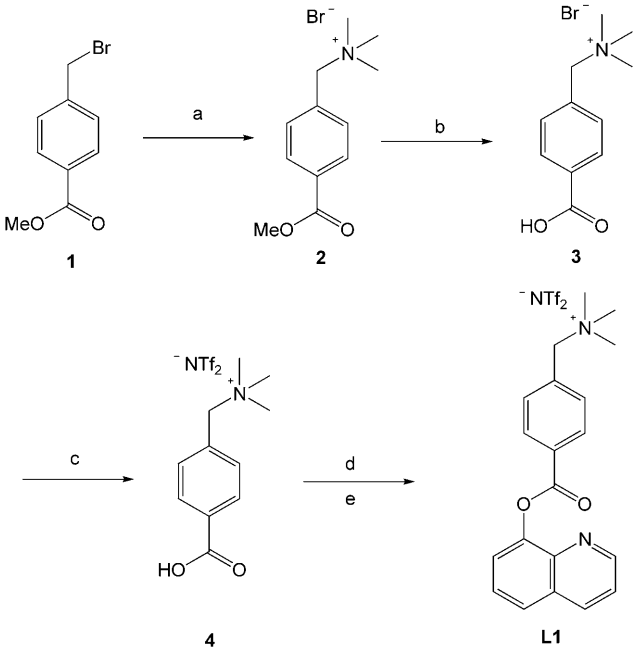
decrease the risk of loss into the aqueous phase compared to classical ligands.

We thus designed and prepared TSOS **L1** (Scheme 1), in which an 8-hydroxyquinoline benzoate complexing/sensing unit for mercury cations is grafted onto an ionium salt. A very



Scheme 1. Synthesis of the TSIL **L1** (see the Supporting Information). a) Trimethylamine, acetonitrile, 80 °C, 15 h; b) NaOH 1 M, 80 °C, 15 h, then HBr 2 M, room temperature, 15 min; c) $LiNTf_2$, water, room temperature, 3 h; d) oxalyl chloride, anhydrous THF, 0 °C, 15 min, then room temperature, 3 h; e) 8-hydroxyquinoline, freshly distilled triethylamine, anhydrous THF, room temperature, 4 h.

hydrophobic counteranion was selected to confine the **L1**-Hg^{2+} complex in the IL phase. The heavy-metal detection is based both on its transfer from a flowing carrier aqueous solution to a storage ionic liquid phase and on the onset of fluorescence emission of **L1** exclusively upon metal binding (Figure 1). Such an "off–on" sensing response to metal-ion binding decreases the likelihood of false positive results and is expected to improve the detection limit owing to the very low offset signal.

First, the absorption characteristics of **L1** were investigated in different solvents. The UV/Vis absorption spectra of **L1** are characterized by two maxima at 227 and 277 nm in acetonitrile, corresponding to a π–π* transition and to a lower-lying *n*–π* transition with intramolecular charge-transfer character.[11] Extinction coefficients ε_{227} and ε_{277} were determined in acetonitrile to be 44400 and 6700 $M^{-1}cm^{-1}$, respectively. The two absorption maxima (λ_{abs}) were found to be almost identical in acetonitrile and in the different studied hydrophobic ionic liquids ([bmim][NTf_2], [bmp][NTf_2], and [tmba][NTf_2]; bmim = 1-butyl-3-methylimidazolium, bmp = butylmethylpyrrolidinium, tmba = *N*-trimethyl-*N*-butylammonium, NTf_2 = bis(trifluoromethylsulfonyl)imide). The fluorescence spectra of the free ligand **L1** in acetonitrile and in the different ionic liquids show very weak fluorescence,

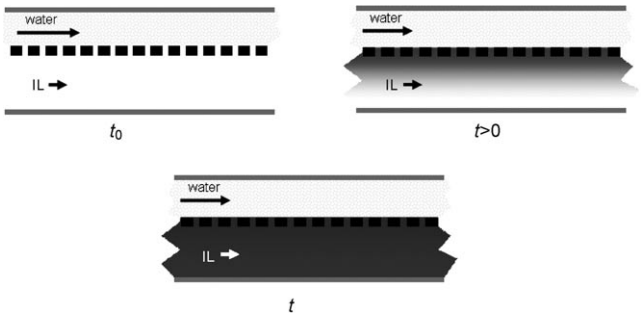


Figure 1. Principle of the real-time monitoring of a liquid–liquid extraction using a fluorogenic task-specific ionic liquid. The two immiscible phases, containing Hg^{2+} ions and TSIL **L1**, respectively, flow in parallel (separated by micropillars). The extraction induces fluorescence, the intensity of which is directly correlated to the generation of the complex [**L1**-Hg^{2+}]. Progressively, the Hg^{2+} ions are extracted by the TSIL **L1** at the liquid–liquid interface, which becomes fluorescent. At the end of experiment, the complex [**L1**-Hg^{2+}] diffuses throughout the channel, which becomes totally fluorescent.

with an emission band peaking at 380 nm and corresponding low quantum yields ($\Phi_0 = 0.002$ in acetonitrile). Such low quantum yields were observed earlier with 8-hydroxyquinoline (8-HQ) benzoate derivatives in which the carbonyl oxygen lone pair is brought into close proximity with the 8-HQ fluorophore.[12]

Next, the ability of **L1** to complex Hg^{2+} ions was checked in different solvents. As shown in Figure 2a, the addition of

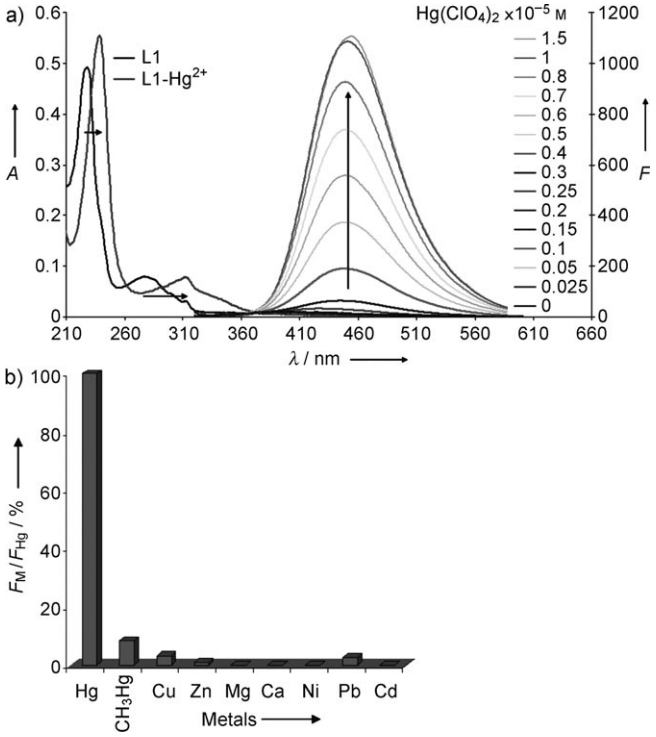


Figure 2. a) Absorption (*A*) and fluorescence (*F*) emission spectra (excitation at 313 nm) of **L1** (10 μM) free and in the presence of increasing concentrations of $Hg(ClO_4)_2$ in [bmp][NTf_2]. b) Selectivity tests for **L1** in [bmp][NTf_2] with Hg^{2+}, CH_3Hg^+, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}

increasing amounts of $Hg(ClO_4)_2$ to a solution of **L1** in [bmp][NTf_2] induces a bathochromic shift of both the high-energy absorption band (shifted to 238 nm) and of the low-energy band (shifted to 313 nm) and the progressive rise of a new intense emission band peaking at 448 nm. At saturation, a maximum fluorescence enhancement factor (FEF) of 94 and a fluorescence quantum yield of 0.41 in [bmp][NTf_2] were determined for the complex **L1**-Hg^{2+}. These major changes can be ascribed to the suppression of effective nonradiative deactivation processes through a $^3(n$–$\pi^*)$ state by lowering the energy of the emitting π--π^* excited state with intermolecular charge-transfer character, thus preventing efficient intersystem crossing.[12]

The selectivity of **L1** for Hg^{2+} cations was evaluated by testing the response to other environmentally relevant metal ions, which provided evidence of high selectivity towards mercury. A maximum fluorescence signal 29-fold smaller than for Hg^{2+} was obtained for Cu^{2+} ($\lambda_{em} = 448$ nm), while no significant fluorescence was observed with Cd^{2+}, Mg^{2+}, Ni^{2+}, and Ca^{2+}. The selectivity ranking was determined to be $Hg^{2+} \gg Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+} \approx Ni^{2+} \approx Mg^{2+} \approx Ca^{2+}$ (Figure 2b). However, Ca^{2+} and Mg^{2+} usually are present in excess over mercury (at least 1000-fold) in water. To evaluate the potential interference of these two species in relevant conditions, **L1** was tested with 2×10^{-3} M Ca^{2+} and Mg^{2+} ions. No signal was observed, showing that these ions cannot fake the presence of Hg^{2+}. Furthermore, the addition of Ca^{2+} and Mg^{2+} ions in 1000-fold excess over Hg^{2+} showed a decrease by only 10% of the Hg^{2+}-induced fluorescence signal obtained in the absence of these two ions. Next, the response of **L1** to CH_3Hg^+, a highly toxic form[13] that is only weakly soluble in water, was determined. A mercury-induced fluorescence signal 12-fold smaller than that obtained for Hg^{2+} under comparable conditions was obtained.

Finally, an experimental detection limit of 10^{-6} M was measured for Hg^{2+}, relatively close to the theoretical detection limit, which is evaluated to be about 2×10^{-7} M, taking into account a signal-to-noise ratio of 3.

After this study conducted in pure IL solvents, macroscopic-scale liquid–liquid extraction experiments were performed using **L1** in [bmp][NTf_2], chosen because of its negligible absorption and fluorescence at operating excitation and emission wavelengths. The extraction of Hg^{2+} ions from aqueous solution was easily performed by shaking, resulting in the formation of a fluorescent ionic liquid phase. The Nernst distribution coefficient was determined to be 13.6 and the extraction yield was 93%. The result was confirmed by ICP-MS.

A proof-of-principle test of liquid–liquid extraction was then conducted in microsystems using TSOS **L1** (10^{-4} M) in [bmp][NTf_2] solution as the binary IL storage phase (see detailed description and fabrication of the microdevice in the Supporting Information). The aqueous solution containing the Hg^{2+} ions was introduced at 4 μL min^{-1} into the 108 μm wide channel. Next, the solution of **L1** in [bmp][NTf_2] was introduced at 0.02 μL min^{-1} into the 150 μm wide channel. When the filling was completed, optical acquisitions started at 450 nm ($\lambda_{exc} = 310$ nm) through a Pyrex plate with an epifluorescence microscope equipped with a UV-LED crown. The two flows were maintained for the duration of the experiment. Figure 3a shows typical fluorescence images of the microsystem during the liquid–liquid extraction. The

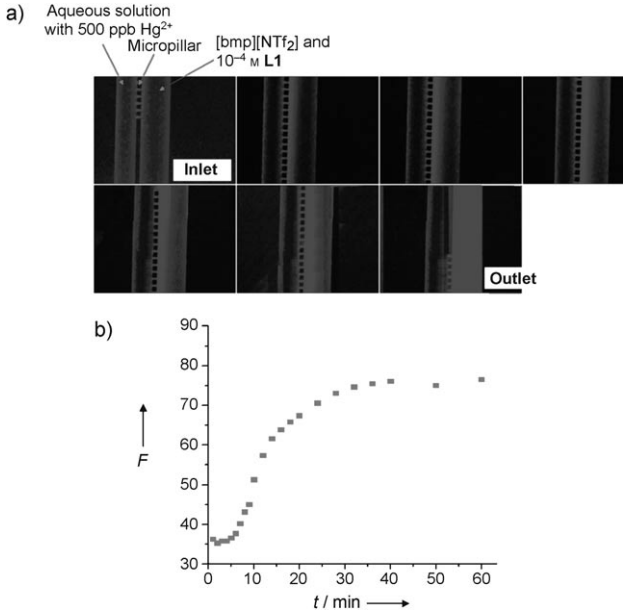


Figure 3. a) Fluorescence intensity monitored at different positions in the microsystem during a liquid–liquid extraction operation (after one hour). The aqueous solution contained 500 ppb $Hg(ClO_4)_2$, and the ionic liquid [bmp][NTf_2] contained 10^{-4} M **L1**. $\lambda_{exc} = 310$ nm and $\lambda_{em} = 450$ nm; b) Fluorescence intensity (F) of **L1**-Hg^{2+} at the microdevice outlet in the ionic liquid phase as a function of time. $t_{exp} = 1$ s, $\lambda_{exc} = 310$ nm, $\lambda_{em} = 450$ nm. The first measurement ($t = 1$ min) gave a relatively high value (37 a.u.), as extraction starts during the filling. When the acquisition was started at the end of the filling, a portion of the **L1**-Hg^{2+} complexes formed during the filling are observed at the microdevice outlet.

fluorescence is observed exclusively in the channel containing the ionic liquid phase with intensity decreasing from the pillars to the opposite wall and increasing from the inlet to the outlet of the microsystem. This observation illustrates the complexation of Hg^{2+} ions by the nonfluorescent ligand **L1** leading to the formation of the fluorescent complex **L1**-Hg^{2+} and its very slow diffusion (10^{-11} m^2 s^{-1})—owing to the high viscosity of [bmp][NTf_2] (85 cP at 25 °C)—away from the interface into the bulk ionic liquid phase. Indeed, the Peclet number (Pe) of the ionic liquid flow being relatively small ($Pe = 222$), the fluorescent complex diffuses with a mass flux only slightly affected by convective transport. The homogeneous fluorescence obtained at the outlet of the microsystem allowed us to monitor the extraction as a function of time (Figure 3b) and to quantify it using a calibration curve. By progressively decreasing the Hg^{2+} concentration in the initially injected aqueous solution, an experimental detection limit of 50 ppb was obtained.

In conclusion, we have described a microsystem prototype allowing for the real-time detection of mercury in water with a very high sensitivity reaching a detection limit of 50 ppb. The proposed technology relies on the combination of several innovations, including a microdevice specifically designed for

liquid–liquid extraction and a binary task-specific ionic liquid including a TSOS bearing a 8-hydroxyquinoline benzoate unit as the extracting/sensing moiety and having the capacity to concentrate the mercury(II) cation in the hydrophobic ionic liquid phase, thus increasing the detection sensitivity. This fluorescent probe is highly selective towards mercury versus other heavy metals such as copper, lead, and cadmium (Figure 2b). Another interesting feature is that the ligand emits fluorescence exclusively when complexed with mercury, therefore providing an "off–on" sensing response. These results clearly open the way towards efficient devices for the real-time tracking of heavy metals in water. Such devices can also be extended to the detection of other pollutants. Collaborative research along these lines is currently in progress.

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