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PAPER

A novel nylon membrane–rhodamine 6G spirocyclic phenylthiosemicarbazide derivative system as a fluorimetric probe for mercury(II) ion†

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A highly sensitive and selective probe for the fluorimetric determination of mercury ion traces in aqueous solution is proposed. The probe is based on the mercury-promoted ring opening of the spirolactam moiety of a rhodamine 6G spirocyclic phenylthiosemicarbazide derivative (FC1) retained in nylon membranes. It is demonstrated that the chemodosimeter preserves its sensor ability, displaying intense fluorescence in the presence of Hg(II) after being immobilized on the nylon surface and reacting with the mercury ion solution *via* a simple syringe procedure. The advantages of this proposal are: (1) the use of an easily affordable solid support which is able to immobilize the FC1 molecular probe without involving a covalent bond, (2) the consumption of a very small volume of organic reagent, dramatically reducing the environmental impact, and (3) the development of a solid phase system potentially useful as a main component for designing chemical sensors capable of providing continuous real-time information. In order to obtain higher and stable fluorescence signals, both experimental and instrumental variables were optimized. Thus, a simple and sensitive fluorescence method for the determination of mercury ion was established. The limit of detection calculated according to 1995 IUPAC Recommendations was 0.4 ng mL^{-1} (lower than the toxic levels in drinking water for human consumption, established by several regulatory agencies), the relative standard deviation was 2.3% ($n = 6$) at a level of 3.5 ng mL^{-1}, and the sampling rate was about 15 samples per hour. The study of the potential interference from common cations demonstrated a remarkable selectivity for the investigated metal ion. The viability of determining Hg(II) ion residues in real water samples was successfully evaluated through the recovery study of several spiked environmental water samples from different locations.

Introduction

Mercury is a ubiquitous pollutant which is present in the environment in different species. Hg(0) reaches the atmosphere through both natural (evaporation from soil and water surfaces and volcano emissions) and anthropogenic sources (emissions from coal-burning power stations, solid waste incinerators, gold mining, *etc.*).[1,2] Mercury vapor is converted to a soluble Hg^{2+} form and returned to the earth in rainwater. Inorganic mercury can either be converted back by microorganisms to the elemental form and reemitted into the atmosphere, or be subjected to microbial conversion to a methyl mercury complex in the aquatic environment.[1] Thus, mercury can be introduced into the human body by vapor inhalation (principally in the Hg(0) form), through the consumed water (as inorganic Hg^{2+}), or eating contaminated fish products (mainly as a methyl mercury complex).[3] Although toxicity varies with the form of mercury, dose, route of ingestion, and with the exposed organism species,[4] all forms have adverse effects on health, justifying the efforts of regulatory agencies to control their presence in environmental samples.[5]

Specifically for inorganic mercury, the United State Environmental Protection Agency (EPA)[6] has set a maximum contaminant level in drinking water of 2 ng mL^{-1}, while the European Union[7] indicates for this ion a value of 1 ng mL^{-1}, highlighting the necessity of developing sensitive methods for its determination. The more frequent methods for determining mercury at trace levels are cold vapor atomic absorption[8,9,10] and fluorescence spectroscopies,[11] X-ray fluorescence spectroscopy,[12] inductively coupled plasma-mass spectrometry,[13] and voltammetry.[14] In the last years, special attention has been paid to the use of electrochemical and optical probes and sensors for selective and sensitive routine monitoring of mercury in water and biological samples.[15–32]

Different research groups have reported revisions of recent progress in the field of optical chemodosimeters and

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chemosensors for chemical and biochemical sensing, including detailed descriptions of those used for mercury determination,[33–39] which give an idea of the recent efforts for developing new colorimetric and luminescence molecular probes and sensors for important environmental pollutants. Among the compounds used as fluorophore probes, rhodamine derivatives have attracted notable interest due to the excellent photophysical properties of the generated products by reaction with specific metal ions such as Cu(II), Pb(II), Fe(III), and Hg(II), among others.[25,40–45] Beija *et al.* have reviewed new synthetic procedures for the preparation of rhodamine derivatives and their applications as fluorescent probes for the detection of selected metal ions and other analytes of interest.[46] One of these rhodamine 6G derivatives, a spirocyclic phenylthiosemicarbazide named FC1, works as a highly selective and sensitive chemodosimeter for Hg(II) ion in aqueous solution.[47] The mercury ion promotes an oxadiazole-forming reaction of FC1, producing a pink product with a strong fluorescence signal in a 1 : 1 stoichiometric manner to the amount of metal ion present. This system was first used as a real-time method for monitoring mercury ions in living cells,[48] and then it was applied for the metal ion determination in water and fish samples.[24] In the latter case, the determination approach was conducted in a water–methanol medium (80 : 20, v/v).

With the purpose of minimizing the environmental impact, there is special interest in developing methods easily adaptable to green chemistry principles.[49] Analytical methods based on solid-phase spectroscopy (SPS) are among those that dominate these principles.[50] Further, SPS plays an important role in the development of probes for the construction of optosensors, with their concomitant advantages of automation and speed.[50] Therefore, in the present work a solid-phase strategy using a commercial nylon membrane as support for the Hg(II)–FC1 interaction is proposed.

Nylon membrane is a polyamide film usually employed for filtering purposes. However, less widespread is its outstanding property as a support material for luminescence generation from selected analytes retained on its surface.[51–55] In the present study, nylon is applied for FC1 retention, and the reaction with mercury occurs on the surface when the metal ion passes through the membrane *via* a syringe procedure (Fig. 1). The study is carried out by analysing the different variables which have influence on the fluorescence intensity of the formed product retained in the nylon membrane. A comparison with other recently proposed sensors and probes is performed, and the feasibility of determining Hg(II) ion in real water samples is demonstrated.

Experimental

Reagents and solutions

The synthesis of the rhodamine 6G derivative (FC1) and the conformation of its structure by ^{1}H NMR and ^{13}C NMR were performed as indicated in ref. 24. $Hg(NO_3)_2 \cdot H_2O$, methanol, acetonitrile, nitric acid and HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) were purchased from Merck (Darmstadt, Germany). Inorganic salts tested as potential interferents were of analytical grade and were used as received.

Nylon membranes (0.2 μm pore size) were obtained from Varian (Seattle, USA), Schleicher–Schuell (Dassel, Germany) and GE Osmonics (Trevose, USA).

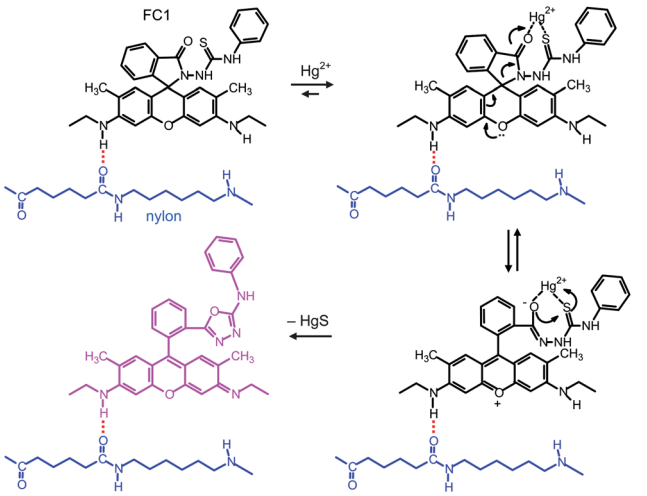

Fig. 1 Scheme of the mercury(II)-induced ring opening reaction and cyclization of the rhodamine 6G derivative (FC1) adsorbed onto nylon.

Stock solutions of FC1 (3.00×10^{-4} mol L^{-1}) were prepared in both methanol and acetonitrile. From these solutions, more diluted solutions were obtained in the corresponding solvent.

A Hg(II) stock solution (about 3400 μg mL^{-1}) was prepared by dissolving mercury(II) nitrate in doubly deionized water containing a few drops of concentrated HNO_3, and the exact concentration of the metal ion was determined by titration with standard sodium chloride and an appropriate indicator. More dilute sample solutions were prepared daily by appropriate dilution of the stock solution with 0.025 mol L^{-1} HEPES buffer solution (pH = 7). Ultrapure water was provided using a Millipore Milli-Q system (Millipore, Bedford, MA).

Special care was taken in the preparation and handling of solutions and containers to minimize any possible risk of Hg^{2+} contamination. Calibrated flasks were left overnight in 10% (v/v) HNO_3 and rinsed with ultrapure Milli-Q water to eliminate contamination before use.

Instrumentation

Fluorescence spectra were measured using an Aminco Bowman (Rochester, NY, USA) Series 2 luminescence spectrometer

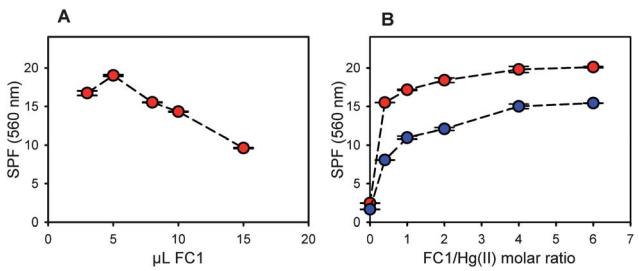

Fig. 2 Solid-phase fluorescence (SPF) intensity measured at 560 nm after filtering 10.0 mL of 5.00 ng mL^{-1} (2.49×10^{-8} mol L^{-1}) Hg^{2+} through: (A) nylon membranes doped with different volumes of 2×10^{-4} mol L^{-1} FC1 dissolved in acetonitrile and (B) nylon membranes doped with 5 μL of different concentrations of FC1 dissolved in methanol (blue circles) and acetonitrile (red circles). λ_{ex} = 505 nm. Error bars correspond to duplicates.

equipped with a 7 W pulsed xenon lamp. These spectra were obtained using excitation and emission wavelengths of 505 and 560 nm, respectively, and both the excitation and emission slit widths were of 4 nm. The photomultiplier tube (PMT) sensitivity was fixed at 320 V and the temperature of the cell compartment was kept constant at 20 °C by circulating water from a thermostatted bath (Cole-Parmer, Illinois, USA). Images were digitized in a Chromadoc-IT (Upland, CA, USA) system using a 5.1 megapixel digital Olympus camera (Tokyo, Japan).

General procedure for fluorescence measurements

All tested nylon membrane brands showed similar behavior related to both background emission and luminescence properties of the fluorescent product. None of these membranes required any conditioning and were used as received.

A typical procedure under the evaluated optimal conditions was conducted as follows: a 13 mm nylon membrane was spotted with 5 μL of 2×10^{-4} mol L^{-1} FC1 solution with the aid of a micropipette and was dried for a few seconds on a plate at about 75 °C. Then, the disk was loaded into a stainless steel filter syringe kit and placed into a 10 mL syringe. In order to increase the sensitivity of the method, the area of the nylon surface was restricted with a polytetrafluoroethylene (PTFE) ring fitted over the membrane before the interaction with Hg(II) ion. Thus, a nylon surface with a final diameter of 5 mm was exposed to the flowing solution. The aqueous mercury ion solutions were forced to pass through the membrane in approximately 1 min per sample. The excess of liquid was purged by forcing volumes of air through the disk with a 20 mL syringe. The membrane was then removed from the stainless-steel head and dried in the heating plate at about 75 °C for one minute. It was verified, through the quality of the subsequent fluorescence measurements and the good relative standard deviation value (see below), that the stages of drying at the indicated temperature did not have detrimental effects either on the fluorescence signal or on the nylon membrane. The nylon disk was then placed in a laboratory-made solid-support holder,[52] consisting in a metallic chamber covered with a low luminescent paint, with a hole where the disk is held in an optimized position with respect to the incident beam and the fluorescence spectra of the fluorescent product retained in its surface were collected at 90° under the instrumental conditions indicated above. A new membrane was used for each analysis.

Real water samples

Samples were collected from the following locations: (1) a river water sample from Paraná River (Rosario, Santa Fe, Argentina), (2) underground water samples from Funes and Venado Tuerto cities (Santa Fe, Argentina), and (3) a spring water sample from the hydrogeological basin of Tunuyán (Mendoza, Argentina). Because these samples did not contain Hg(II) ion at levels higher than the attained detection limit, a recovery study was carried out by spiking them with the metal ion at two different concentrations. While both mineral water and the underground water from Venado Tuerto underwent no previous treatment, both the river water sample and the underground water sample from Funes were filtered through a nylon membrane after the addition of the analyte and before carrying out the corresponding measurement. This was done in order to mimic a real situation for a sample containing mercuric ions, thus demonstrating that the analyte is not lost during filtration. The mercury concentration in real samples was corroborated by cold vapor-atomic absorption spectroscopy following the EPA 7470A method.[56]

Results and discussion

Solid-phase fluorescence strategy

As already stated, the reaction between FC1 and Hg(II) has been previously reported to occur in fluid solution.[24,47] The strategy for saving both the synthetic reagent FC1 and the organic solvent in which it is dissolved consisted in adsorbing a few microlitres of FC1 solution in a nylon membrane, allowing the reaction with mercury ion (Fig. 1) to occur with the FC1 molecule attached to the solid support, generating the fluorescent product. It should be noted that the amount of FC1 consumed in each experimental run following the proposed approach (0.55 μg) is significantly smaller than that used in the measurement carried out in solution using a 3 mL conventional cell (16.6 μg).[24]

A possible explanation for the FC1 retention mechanism on the nylon surface is the formation of hydrogen bonds between carbonyl oxygens in nylon and the secondary amine groups of FC1. In this way, the spirolactam moiety of the rhodamine 6G derivative is free for reacting with Hg(II) ions, producing the fluorescent product which is measured on the solid surface (see below). No detectable leaching of FC1 in the eluted aqueous solution was observed, indicating that the adsorption mechanism of FC1 is efficient enough to avoid significant loss when the Hg^{2+} ion solution is filtered through the disk. This fact is also clearly demonstrated by the final fluorescent product obtained. Furthermore, since the reaction is irreversible, a new disk with fresh reactive surface is used in each measurement, and therefore the sensing capability of the probe remains unaltered. In fact, the reproducibility of the obtained spectra indicates that the adsorption mechanism in nylon provides a robust strategy with a minimum experimental effort and a very short experimental time (about 4 minutes total per sample).

It is important to mention that the immobilization of a sensor molecule on the solid support frequently involves a covalent bond, providing the possibility to regenerate and reuse the probe without the risk of diminishing its sensor capability. However, time and effort are certainly involved in the preparation and optimization of these types of probes. In this way, it is interesting to note that in recently reported solid supported sensors, based on rhodamine derivatives molecular probes, more than 30 min are needed for the detection of Cu(II) by immobilizing a rhodamine derivative on a ultrathin platinum film[42] or for the detection of Fe(III) by immobilization of a rhodamine derivative in a polyvinyl alcohol (PVA) polymer film.[43]

Selection of the solid-phase fluorescence experimental conditions

With the purpose of using a very low amount of organic reagent, the selection of the volume of FC1 solution to be spotted over the nylon disk was performed in the range 3–15 μL. The results (Fig. 2A) showed that a volume of 5 μL of 2×10^{-4} mol L^{-1} FC1 in acetronitrile solution was the most favorable one to deposit

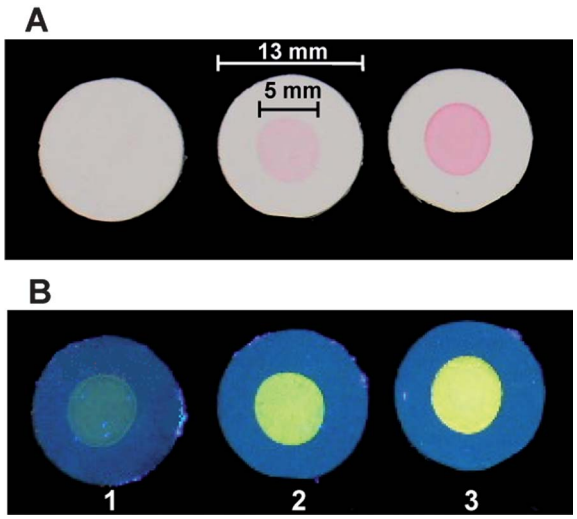

Fig. 3 Photographs of FC1 doped nylon membranes irradiated with visible (A) and UV (B) lamps, after treatment with 10 mL of: (1) Hg^{2+}-free water, (2) 5 ng mL^{-1} Hg^{2+} solution, and (3) 12 ng mL^{-1} Hg^{2+} solution. The bars indicate the diameters of the nylon membrane and of the area exposed to the solution flow.

the reagent over the nylon surface. Lower volumes do not properly cover the membrane, and higher volumes produce a detrimental effect in the subsequently measured fluorescence.

Both the best solvent for dissolving FC1 and the suitable equivalents to be deposited on the nylon surface were determined passing the same mercury solution through nylon membranes treated with increasing amounts of FC1 dissolved in either methanol or acetonitrile. Solvents play an important role in solid-phase fluorescence, through a competitive effect with the analyte retention and also through their influence on the quantum yield of the immobilized fluorescent compound.[57] As can be observed in Fig. 2B, the fluorescence signal of the formed product is higher when FC1 is dissolved in acetonitrile and, therefore, this solvent was selected for depositing FC1 on the nylon disk. Fig. 2B also shows a fluorescence intensity saturation at stoichiometries higher than 1 : 1 FC1/Hg(II), similar to the behavior in aqueous solution.[24] Thus, five microlitres of a 2×10^{-4} mol L^{-1} FC1 acetonitrile solution were used in the experiments performed with 10.0 mL of mercury(II) solution (see below), which guarantees an adequate FC1/metal ion ratio in the investigated concentration range.

In relation to the volume of mercury solution filtered through the nylon disk, it is well-known that in those methods involving solid-phase retention, sensitivity can be improved by applying higher sample volumes.[58] However, when nylon membranes are used as an extractive support, volumes higher than about 50 mL can produce clogging problems, and the analysis time made the experiment impractical.[59] Volumes from 1 to 20 mL of mercury solution were tested, and a value of 10 mL showed to be appropriate for observing a significant signal without involving a large experimental time.

The study of the influence of the pH on the fluorescence profile of the investigated system showed a behavior similar to that found in solution: FC1 responds to Hg^{2+} in the range from pH 5.5 to 12.0, where the fluorescence of FC1 is almost negligible.[24] Although fluorescence enhancement in the presence of Hg^{2+} also occurs at lower pH, the luminescence intensity of free FC1 interferes in the determination. Thus, in order to ensure an optimal signal of the fluorescent product, avoiding interference from FC1 itself, the pH of the mercury solutions was fixed at 7 with HEPES buffer. It is interesting to note that the quantitation of Hg^{2+} can be performed in aqueous solutions in a wide pH range.

Details on the selection of the instrumental parameters, including optimization of the photomultiplier tube voltage (Fig. S1), are provided in the ESI†.

Solid-phase spectra

The ability of the nylon membrane to retain FC1 on its surface and to allow the reaction with Hg^{2+} yielding a pink product which is highly fluorescent can be macroscopically appreciated in Fig. 3. This figure displays photographs of three nylon membranes irradiated with both visible (Fig. 3A) and UV (Fig. 3B) lamps after the treatment of FC1 doped nylon membranes with solutions of different mercury concentrations.

Fig. 4 shows the fluorescence excitation and emission spectra of FC1 in the nylon membrane before and after passing through Hg(II) ion aqueous solution at different concentrations. The obtained signals remain almost constant at least for twenty-four hours. The comparison of these spectra on nylon with those in solution[24] shows that the nylon membrane does not promote significant changes in the emission maxima of the fluorescence product.

Performance of the developed probe

A calibration graph was obtained under the established working conditions and the obtained results are shown in Table 1. The data were fitted by standard least-squares regression and the linear relationship between the mercury ion concentration and the fluorescence emission measured was corroborated applying the *F* test recommended by IUPAC.[60] Regarding the obtained limit of detection (LOD = 0.4 ng mL^{-1}, calculated according to 1995 IUPAC Recommendations[61,62]) one may conclude that the

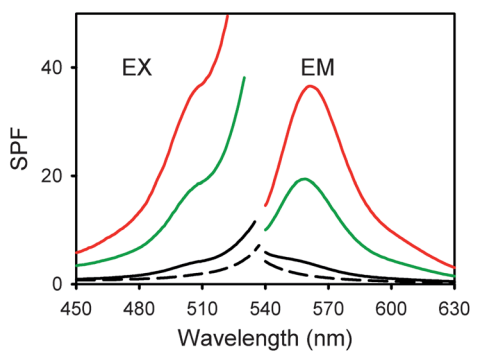

Fig. 4 Excitation and emission solid-phase fluorescence (SPF) spectra of the product obtained after filtering 10.0 mL of Hg^{2+} solutions through nylon membranes doped with 5 μL of 2×10^{-4} mol L^{-1} FC1 acetonitrile solution. From bottom to top: nylon membrane background (black dashed-line), 0 (black line), 5 (green line) and 10 ng mL^{-1} (red line) Hg^{2+} solutions.

Table 1 Analytical parameters[a]

Linear range (ng mL^{-1})	1.2–12.0
Slope[b]	3.31 (0.04)
Intercept[b]	3.5 (0.2)
Correlation coefficient	0.997
(γ^{-1})[c] (ng mL^{-1})	0.2
LOD[d] (ng mL^{-1})	0.4
LOQ[e] (ng mL^{-1})	1.2
RSD[f] (%)	2.3
Sampling rate (samples h^{-1})	~15

[a] The number of data for the calibration curve corresponds to seven different concentration levels (extracted volume = 10 mL), with three replicates for each level (n = 21). [b] The corresponding standard deviations are given in parentheses. [c] The inverse of analytical sensitivity (γ) represents the minimum concentration difference which can be measured. [d] Limit of detection calculated from ref. 61 and 62. [e] Limit of quantitation calculated as (10/3.3) × LOD. [f] Relative standard deviation for a sample containing 3.5 ng mL^{-1} (n = 6).

result herein attained, at sub part-per-billion levels, would allow the analysis of trace Hg^{2+} residues, and thus the detection and quantification of toxic levels of Hg^{2+} in drinking waters for human consumption, according to several regulatory agencies.

In comparison with the limits of detection for selected methods recently reported for Hg^{2+} (Table 2), values from 0.01 to 200 ng mL^{-1} using different strategies have been found. It should be noticed that many of the reported methods in Table 2 involve the preparation of either electrochemical or optical sensors such as selective electrodes, polymer films or plasticized membranes. The best limit of detection (0.01 ng mL^{-1}) was attained with cold vapor atomic absorption spectroscopy, although a preconcentration step with C18 disk modified with a triazine ligand was required. In the present case, a low limit of detection is achieved using a commercial support coupled to a non-sophisticated analytical technique and without applying pre-concentration steps. In addition, a sampling rate of about 15 samples per hour makes the method attractive for routine laboratories.

Interference study

Cations concomitantly present in the analysed samples might react with FC1 in a similar way to the mercury ion (increasing the resulting signal) or produce a quenching effect (decreasing the resulting signal). Therefore, in order to assess the possible analytical application of the method, a systematic study for

Table 2 Comparison of the analytical performance of selected methods recently reported for the determination of mercury ion

Methodology	LOD[a] (ng mL^{-1})	Linear range (ng mL^{-1})	RSD[b] (%)	Ref.
Atomic spectroscopy				
Preconcentration with C18 disk modified with a triazine ligand and cold vapor atomic absorption spectroscopy	0.01[c]	0.02–1.90	2.9 [0.1] 1.1 [1.0]	10
Electrochemistry				
Probe based on an imprinted polymer and graphite	0.1[c]	0.5–1 × 10^2		15
Probe based on a multi-walled carbon nanotubes–ionic liquid–carbon paste electrode with a triazene derivative	0.5[d]	1–2 × 10^4	0.5 [2 × 10^3]	16
Spectrophotometry				
Probe based on hexathiacyclooctadecane and chromoionophore V and a plasticized PVC membrane	40	42–2.4 × 10^4	1.5 [4 × 10^2] 1.9 [4 × 10^3]	17
Probe based on a synthesized ligand[e] and a plasticized PVC membrane	0.02[d]	0.06–6.4 × 10^5	0.02 [200] 0.04 [2 × 10^4]	18
Probe based on a synthesized ligand[e] and a sol–gel film	0.22[d]	0.3–3.4 × 10^6	0.020 [200] 0.026 [2.0 × 10^5]	19
Probe based on a triazine derivative and a plasticized PVC membrane	0.04[c]	0.18–50	3.4 [1.0] 3.1 [10]	20
Probe based on a Schiff's base ligand and an agarose membrane	200.6[c]	2 × 10^3 to 2 × 10^6	2.6 [1 × 10^4] 1.5 [4 × 10^5]	21
Spectrofluorimetry				
Probe based on quenching of a porphyrin derivative immobilized in a plasticized PVC membrane	1.6[c]	8.0–8 × 10^2	4.24 [12] 3.78 [80] 4.02 [4 × 10^2]	22
Use of a porphyrin-quinoline derivative	4.4	60–4 × 10^3		23
Use of a rhodamine 6G derivative	0.7[f]	0–12		24
Probe based on rhodamine 6G grafted onto quantum dots-silica nanoparticles	0.52[c]	8–160	3.9 [100]	25
Probe based on 1-amino-8-naphthol-3,6-disulfonic acid intercalated layered double hydroxide film	12.6	20–2 × 10^3	Less than 3	26
Spectrofluorimetric probe based on a triazine–thione derivative and a plasticized PVC membrane	0.036[c]	0.1–1 × 10^4		27
Probe based on quenching of a mesoporous silica with a 1,8-naphthalimide-based receptor	200			28
Probe based on quenching of a benzoxadiazole–thiourea conjugate	120[c]	0–2 × 10^3		29
Probe based on a rhodamine 6G derivative bearing a thiolactone moiety		0.2–2		43
Probe based on a rhodamine-derived Schiff base		100–2 × 10^3		45
Probe based on a rhodamine 6G derivative and nylon membrane	0.4[f]	1.2–12.0	2.3 [3.5]	This work

[a] Limit of detection. [b] Relative standard deviation for the concentrations [ng mL^{-1}] given in brackets. [c] Calculated as three times the standard deviation of the blank divided the slope of the calibration curve. [d] Obtained from the intersection of the two segments of the calibration graph of the response at its lowest part. [e] 4-Phenyl-2,6-bis(2,3,5,6-tetrahydrobenzo[*b*][1,4,7]trioxononin-9-yl)pyrylium perchlorate. [f] Calculated from ref. 61 and 62.

Table 3 Recovery study of Hg(II) ion for spiked water samples

Sample	Taken (ng mL^{-1})	Found (ng mL^{-1})[a]	CV–AAS[b]
Mineral water[c]	5.00	5.26 (0.09)	5 (1)
	3.00	3.04 (0.05)	3.4 (0.1)
Underground water[d]	2.00	1.99 (0.02)	2 (1)
	5.00	4.99 (0.12)	5 (2)
Underground water[e]	5.00	5.01 (0.07)	5.5 (0.3)
	2.50	2.50 (0.01)	2.8 (0.1)
River water[f]	1.50	1.59 (0.02)	1.2 (0.1)
	5.00	4.99 (0.06)	5.1 (0.1)

[a] Mean of duplicates; standard deviation is given between parentheses. [b] Cold vapor-atomic absorption spectroscopy as a reference method;[56] mean of duplicates; standard deviation is given between parentheses. [c] From the hydrogeological basin of Tunuyán (Mendoza, Argentina); this water contains Ca(II) (30 μg mL^{-1}), Mg(II) (3 μg mL^{-1}), Na(I) (10 μg mL^{-1}), K(I) (4 μg mL^{-1}), HCO_3^- (79 μg mL^{-1}), SO_4^{2-} (44 μg mL^{-1}) and F^- (1.2 μg mL^{-1}). [d] From Funes City surroundings (Santa Fe, Argentina). [e] From Venado Tuerto City surroundings (Santa Fe, Argentina). [f] From the Paraná River (Santa Fe, Argentina).

detecting interferences was undertaken. This involves adding known amounts of each potentially interfering ion to a solution containing the analyte. If interference effects occur, the concentration of the foreign ion is progressively reduced until the effect is not significant, within a specified tolerance.

The foreign ions assayed were Mn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Bi^{3+}, Zn^{2+} and Ag^+, at concentrations 100-times higher than mercury ion (5.0 ng mL^{-1}). Tolerance was estimated as ±6.9% in the determination of Hg(II), which represents three times the obtained relative standard deviation (RSD = 2.3, see Table 1). Among the investigated cations, only Ag(I) produced a very slight interference (tolerated Ag^+/Hg^{2+} ratio = 10), possibly by reaction with FC1, when it is present at concentrations ten times higher than Hg(II) ion. The remaining investigated cations did not influence the measured signal.

Real samples

Hg(II) ion determination under the established conditions was carried out in spiked real matrices of different water types. Table 3 shows that the results provided by the proposed strategy are similar to those obtained with a reference method. The statistical comparison between both methods was carried out by the so-called elliptical joint confidence region (EJCR) test.[63] This test is recommended for checking the accuracy of a method, and consists of plotting the region of mutual confidence (usually 95%) of the slope and intercept for the plot of predicted *vs.* nominal concentrations in the plane slope-intercept. The region has an elliptical shape, and the test checks whether the theoretically expected values of slope = 1 and intercept = 0 are included within the ellipse. As can be seen in Fig. S2 of the ESI†, the ideal point is included in both ellipses, indicating that both proposed and reference methods are comparable in their analytical predictions.

Additionally, the amount of mercury ion recovered with the proposed method was compared with the concentration added to each sample through a paired *t* test.[64] Using the data shown in Table 3, the experimental *t* value obtained was 1.04, whereas the critical $t_{\alpha,\nu}$ ($\alpha = 0.05$, $\nu = n - 1 = 7$) was 2.36. Therefore, since the experimental *t* value is lower than the critical one, there are no statistical differences between both found and nominal concentrations.

The results also suggest that interference from the background of the investigated samples (ions and organic compounds) is absent.

Conclusion

In summary, immobilization of FC1 on a nylon membrane resulted in a highly efficient optical luminescent probe for Hg(II) ion determination. Nylon is demonstrated to be a suitable support for our purposes, allowing the reaction induced by the presence of mercuric ion to occur on its surface. In this way, the proposed method saves organic reagents (both the synthesized FC1 and the solvent where it is dissolved) in relation with the solution system, preserving (or improving) relevant analytical properties such as sensitivity, selectivity and rapidity. Through the simple and inexpensive proposed methodology, mercury ion was successfully determined at trace levels in environmental water samples from different locations.

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