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An optical sensor for mercuric ion based on immobilization of Rhodamine B derivative in PVC membrane

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ABSTRACT

A novel prepared mercuric ion PVC membrane sensor based on a Rhodamine B derivative (RND) as a selective sensing material was described. The sensor exhibited a specific fluorescent off-on response to Hg^{2+}, and showed a linear response over the Hg^{2+} concentration range from 1.0×10^{-9} to 2.0×10^{-3} M with a very low detection limit of 8.1×10^{-10} M in bulk method. The sensor has also been incorporated into a flow-cell for determination of Hg^{2+} in flowing streams with improved sensitivity and detection limit. The sensor shows excellent selectivity toward Hg^{2+} with respect to common coexisting cations. The proposed fluorescence optode was successfully applied to detect Hg^{2+} in environmental water samples and fish.

1. Introduction

Natural phenomena and human activities, including volcanic eruptions, wind erosion, water erosion, solid waste incineration and industrial production, can result in mercury pollution of the environment [1,2]. Mercury and its derivatives are highly toxic pollutant. Inorganic mercury can be converted into methyl mercury by bacteria, and easily accumulates in mammals by entering the food chain. When absorbed in the human body, mercury leads to damage to the brain, kidneys and endocrine system [3–5]. Therefore, the detection of mercury becomes increasingly important in the aspect of both environment and human health.

Concern over the distinct toxicity of mercury has stimulated explorations aimed at developing cost-effective, rapid, and facile methods to monitor mercury in different biological, industrial and food samples [6–8]. Compared with conventional techniques [9–18], such as atomic absorption/emission spectroscopy, inductively coupled plasma mass spectrometry (ICPMS), selective cold vapor atomic fluorescence spectrometry, X-ray microanalysis and variety potentiometric ion-selective electrodes, chemical sensors based on optical signal measurement are considered as the advanced techniques because of its simplicity, reasonable selectivity, improved sensitivity, low detection limit and fieldwork applicability. As such, there has been a growing need or desire for constructing optical chemical sensors (optodes) for fast and economical monitoring of mercuric ion in real time. To date, numerous excellent optodes with different mechanisms have been reported for the detection of mercury [19–27].

Prior to our investigation, Rhodamine B derivatives have attracted considerable interests as excellent off-on-type probes for various metal ions [28–35], and selective Hg^{2+} induced spirolactam ring-opening processes were also observed [36–43]. Generally, polyvinylchloride (PVC) has been used for the preparation of the membrane sensors for Hg^{2+} due to its relatively low cost, good mechanical properties and amenability to plasticization [44]. The different schemes used for the preparation of membrane optodes for Hg^{2+} are: (i) fluorescence quenching of Hg^{2+}-selective ionophore 5,10,15,20-tetraphenylporphyrin (H2tpp) immobilized in PVC [26]; (ii) 1-(dansylamidopropyl)-1-aza-4,10-dithia-7-oxacyclododecane (L) as a neutral Hg^{2+}-selective fluoroionophore in PVC membrane [8]; (iii) 1-(2-pyridylazo)-2-naphthol (PAN) immobilized into PVC [24]; (iv) 2-mercapto-2-thiazoline (MTZ) together with ETH5294 chromoionophore into PVC [27]; (v) triazinethione derivative (EHT) immobilized into polyvinylchloride (PVC) [44]; (vi) fluorescence detection using lipophilic porphyrin dimer DTPP into PVC matrix [53]; (vii) 4-phenyl-2,6-bis(2,3,5,6-tetrahydrobenzo[b][1,4,7]trioxononin-9-yl) pyrylium perchlorate

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as an ionic carrier in a plasticized PVC membrane [52]; (viii) irreversible plasticized PVC optode incorporating a tetraarylborate salt and a porphyrin proton-selective fluoroionophore [45]; (ix) 2-mercapto-pyrimidine (2-MP) together with ETH 5294 in PVC membrane [54] and (x) a fluorescent sensor using 5,10,15-tris (pentafluorophenyl)corrole H_3(tpfc) as fluorophore in PVC [55]. The PVC membrane optode could be simple to prepare and capable of producing visual response in the form of changes in absorption, reflectance or fluorescence behavior of the sensitive layer. However, few precedents exist for the use of fluorescent enhancement-based chemosensors in the PVC membrane phase monitoring systems to detect mercuric ions directly [44,45]. Thus, we describe the first example of a reversible mercuric ions PVC membrane sensor based on a Rhodamine B derivative (RND), in which the unique dual chromo- and fluorescence enhancement was observed.

2. Experimental

2.1. Reagents

The plasticizers, dioctyl phthalate (DOP), tributyl phosphate (TBP) were provided by Meilin (Tianjin); dinonyl phthalate (MSDS) was obtained from B.D.H. (UK); bis(2-ethylhexyl)sebacate (DOS) and 2-nitrophenyloctyl ether (NPOE) were purchased from TCI (Tokyo). Polyvinylchloride (PVC) (high molecular weight) was obtained from Sigma (Italy). Tetrahydrofuran (THF) (Meilin, Tianjin) was used as received without any further purification. Buffer solution (0.05 M) was made by dissolving the appropriate amounts of Tris(hydroxymethyl) aminomethane (Tris) in distilled water and adjusting pH to the desired value with 0.1 M HCl, which was used as a conditioning solution and for preparing all working solutions. A stock solution of 1.0×10^{-2} M Hg^{2+} was prepared by dissolving mercuric nitrate ($Hg(NO_3)_2{\cdot}0.5H_2O$) in 0.01 M nitric acid (HNO_3). Working solutions of mercuric nitrate were freshly prepared by serial dilution of the stock solution with 0.05 M Tris–HCl buffer solution. The selected sensing material (RND) was prepared according to the literature method [46–48] (Scheme S1) and the product was verified by ^{1}H NMR (Fig. S1). As follows: Rhodamine B is connected to 2-hydroxy-1-naphthaldehyde through a hydrazine bridge; that is, a one-step Schiff base reaction. Metal salts used in this study were added as aqueous solutions in distilled water of their corresponding nitrate salts. All other chemicals used were of analytical reagent grade, and double distilled-deionized water was used throughout.

2.2. Apparatus

UV 1100 spectrophotometer (Techcomp, Beijing) was used for absorption measurements. Fluorescence spectra were performed on a Hitachi F-4500 spectrophotometer running the FL Solutions software package. The pH was adjusted with an Orion 720+ pH/Ion Meter (Thermo Electron, USA) equipped with a combined glass electrode.

The flow-through cell consisted of two separate Teflon blocks, optical glass window, sample chamber connected to inlet and outlet Teflon tubes, a thick silicon gasket and screws for clamping the blocks. The sensing membrane was coated on the surface of the glass slide, which formed one of the walls of the chamber. The experimental set-up is shown in Fig. 1. It can be seen from Fig. 1, the incident light was focused on sensing membrane at an angle of 45° and the fluorescence intensity was detected at a 90° angle relative to the incident light. The measuring cell was mounted in the fluorescence spectrophotometer and connected with a peristaltic pump (Chuangrui Instruments, Baoding, China) to obtain the flow-through system.

2.3. Preparation of the optode membrane

Membrane solutions were prepared by dissolving 32.5 mg PVC, 65.0 mg TBP and 2.5 mg RND in 2.0 ml THF. The solution was stirred with a magnetic stirrer to obtain a homogeneous mixture. Glass slides for bulk measurements were cut from microscope slides into 12 mm × 16 mm dimensions to fit precisely into its diagonal width of standard quartz cuvette. To improve the adhesion of the membrane, the glass slides were cleaned with THF, sulfuric acid and

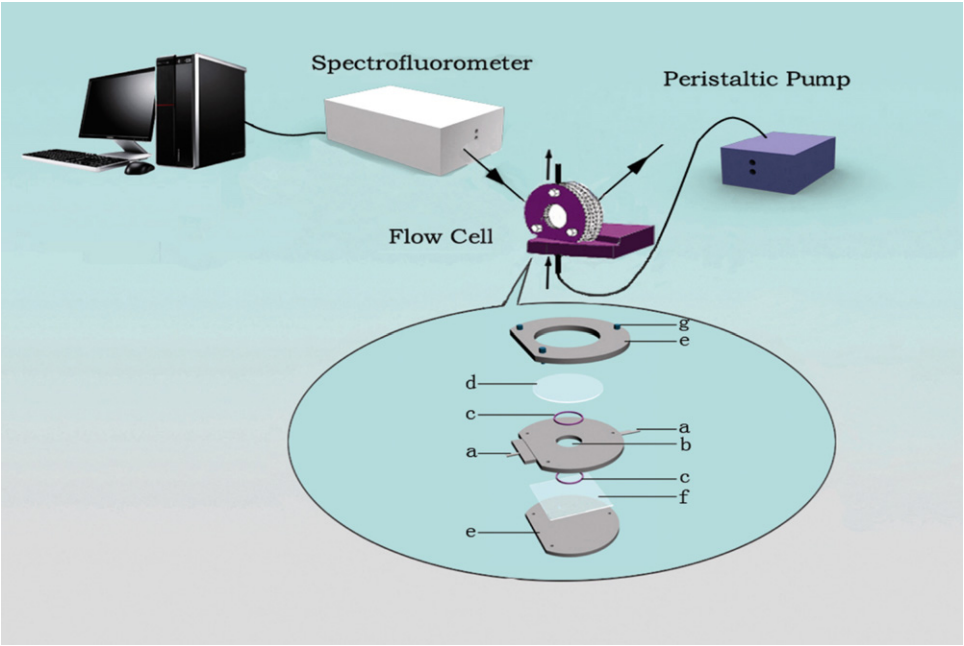

Fig. 1. Schematic diagram of the flow-through cell: (a) Teflon tube for sample in and out; (b) sample chamber; (c) gasket; (d) optical glass window; (e) Teflon cover; (f) sensing membrane coated on the glass slide; (g) fixing screws.

sodium hydroxide solutions, respectively, then thoroughly rinsed with distilled water and finally dried in an oven at 110 °C. Membranes were cast by placing 35 μl of the membrane solution onto the glass slide of ~10 μm thickness and spread evenly using a capillary glass tube [27,54,56]. After 2 min, the coated slides were transferred to a Petri dish with a filter paper cover, and then stored away from light for 12 h before use. The blank membrane (membrane in a buffer solution in the absence of Hg^{2+}) was taken as reference. The coated membrane for the flow-cell was prepared by spreading 30 μl of the membrane cocktail onto the glass slide over an area of ~1.8 cm^2 and stored as above.

2.4. Measurement procedure

Unless otherwise noted, all the measurements were performed by means of the following test. Sample solutions were prepared by mixing with a solution containing different concentration of Hg^{2+} and 0.05 M Tris–HCl buffer solution (v/v, 1:4). Subsequently, Absorption and fluorescence emission spectra of PVC membranes were acquired in quartz cuvettes which were filled with sample solution. The films were placed in diagonal position in the quartz cuvette (detector in 90° in respect to incident light). The advantage of this kind of placement was to improve the reproducibility of the measurements. For fluorescence measurements, excitation was provided at 374 nm, and emission was collected from 550 to 640 nm, the excitation and emission slit widths were 5 nm (Fig. S3). Finally, the membrane was regenerated by immersing it in 0.01 M NaOH (containing 0.1 M NaCl) for a period of 60 min for complete stripping of mercuric ion from the membrane. All fluorescence measurements were made with respect to the blank (reference) membrane in 0.05 M Tris–HCl buffer solution, pH 6.5.

For the flow-through measurements, the conditioning, measurement and regeneration steps were implemented by passing the buffer, mercury and regenerating solutions, respectively, through the flow cell. All measurements were conducted at least in triplicate.

2.5. Sample preparation

All glassware was rinsed with dilute nitric acid and millipore filtered water before use. A weighed sample (100–200 mg) of freeze-dried fish and 5 ml of HNO_3 (65%, v/v) were placed in the digestion vessel. Digesting was carried out using a microwave-assisted extraction system (Speedwave MWS-3, Berghof, Germany) at 20 W for 5 min [49]. After cooling to room temperature, the solution was directly filtered with a 0.45 μm membrane, the pH adjusted to 2.0 and then stored at 4 °C in the dark until analyses. A 0.5 ml aliquots of the final solutions obtained were analyzed for mercury content using the proposed optical sensor by standard addition method.

A water sample from a pond on our campus was filtered through a 0.45 μm membrane, preserved by acidification with HNO_3, and stored in polyethylene bottles and analyzed within 12 h of the collection. All the samples without or with addition of Hg^{2+} at different concentration levels were determined by the proposed sensor.

3. Results and discussion

3.1. Optimization of membrane composition

The membrane composition and the properties of solvent mediators, as well as the PVC/plasticizer weight ratio used had significant effects on the response characteristics of the optode membrane. The influence of the membrane compositions on the response characteristics has been well documented [8,26,27,44,53–55].

Several solvent mediators such as DOP, DOS, TBP, MSDS and NPOE were tested as potential plasticizers for preparing the membrane. The TBP containing membrane gave the best fluorescent enhancement-based response toward Hg^{2+} compared to the other plasticizers (Fig. S4). The influence of TBP and PVC concentration was studied for TBP/PVC wt. ratios of 1:1, 3:2 2:1 and 4:1. The results in Table 1 demonstrated that the detection limit of the sensor increased with the increase of TBP/PVC wt. ratio from 1:1 to 2:1, and response range became wider. But too thin membrane (TBP/PVC wt. ratio of 4:1) would result in mechanical strength losing and easily broken. It could also be found that as the ratio of TBP/PVC was higher than 2:1 (w/w), the physical properties of the membrane became worse, that is, the membrane lost homogeneity and the drying time increased considerably. As a result, a TBP/PVC wt. ratio of 2:1 was selected because at this ratio the membrane had relatively low response time and widest working dynamic range. The prepared mixtures containing different amounts of plasticizer/PVC weight ratios are in accordance with the literatures [26,27,44,53–55].

Several membranes were made with different percentage of RND in the range of 0.5–4.5 wt.% (TBP/PVC wt. ratio of 2:1). The data given in Table 1 is also indicative of the influence of the amount of RND on the working range of the proposed optical sensor. Optimum response was found when the concentration of ligand RND was 2.5 wt.% in terms of the lower limit and the upper limit of the working concentration range. While much higher concentrations of the ligand RND led to lower transparency, possibly because of phase separation inside the membrane. It should be noted that the use of over 4.5% RND in the preparation of membrane sensor caused fluorescence signals decreased considerably with the increasing of Hg^{2+} concentration (Fig. S5). This could be attributed to low transmission of light caused by the intense pink-red color of the membrane.

The experimental results showed that the optode membrane consisting of 32.5% PVC, 65.0% TBP, 2.5% RND (all in wt.%) could

Table 1
Optimization of membrane ingredients.

Membrane number (TBP/PVC[a])	Composition (%)				
	PVC	TBP	RND	Working range (M)	Response time (min)[b]
1 (4:1)	19.5	78.0	2.5	2.0×10^{-9}–2.0×10^{-4}	21
2 (2:1)	32.5	65.0	2.5	1.0×10^{-9}–2.0×10^{-3}	15
3 (3:2)	39.0	58.5	2.5	2.0×10^{-9}–2.0×10^{-5}	3
4 (1:1)	48.7	48.7	2.5	2.0×10^{-8}–2.0×10^{-5}	3
5 (2:1)	33.2	66.4	0.5	2.0×10^{-9}–2.0×10^{-3}	24
6 (2:1)	32.3	64.6	3.0	2.0×10^{-9}–2.0×10^{-4}	9
7 (2:1)	31.8	63.6	4.5[c]	2.0×10^{-9}–2.0×10^{-6}	3

[a] TBP/PVC is wt. ratio.
[b] Response times for 2.0 μM Hg^{2+}.
[c] Fluorescence signals decreased with Hg^{2+} concentration increasing.

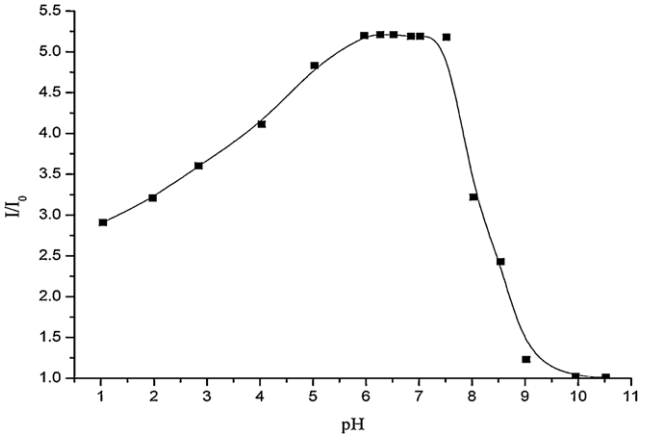

Fig. 2. Effect of pH on the response of the optode in the presence of 2.0 μM Hg^{2+} at 374 nm (374 nm is excitation wavelength).

provide the best response for Hg^{2+}, which was chosen for further experiments.

3.2. Effect of pH

The fresh sensor membranes should first be equilibrated with the conditioning solution prior to obtaining stable fluorescence signals. Fresh membranes showed a very weak response toward Hg^{2+} solutions. Among several conditioning solutions assayed, a 0.05 M Tris–HCl buffer solution was found to be the best choice with a shortest conditioning time of 11 min. The regenerated sensor also showed the same behavior.

The effect of pH of test solution on the response characteristics of the optode was studied by measuring the sensor response to 2.0 μM Hg^{2+} in the pH range 1.0–10.5 using Tris–HCl buffer solutions. The sensor response was expressed as fluorescence intensity ratio I/I_0, which was defined as the ratio between the fluorescence intensity of the Hg^{2+}–RND complex and the fluorescence intensity of the immobilized RND alone at the excitation wavelength of 374 nm and the emission wavelength of ca. 590 nm. pH values were adjusted by the addition of dilute hydrochloric acid. As it was seen from Fig. 2, the fluorescence intensity ratio of the optical sensor first increased with increasing pH value of solution from 1.0 to 6.0, then reached a plateau between pH 6.0 and 7.5, and further decreased at pH > 7.5. The diminished response at pH > 7.5 could be due to a slight swelling of the polymeric film under alkaline conditions, as well as the hydroxide formation of mercuric ions, which resulted in decreased concentration of free Hg^{2+} in sample solution. Thus, in subsequent experiments, a pH 6.5 Tris–HCl buffer solution (0.05 M) was used as an ideal experimental media.

3.3. Response characteristics

Fig. 3 shows the fluorescence emission spectra of the optode membrane on addition of different concentrations of Hg^{2+} in Tris–HCl buffered solutions (pH 6.5). Without Hg^{2+}, free RND membrane displayed very weak fluorescence. After addition of Hg^{2+}, however, noticeable increasing (approximately 5-fold enhancement of I/I_0, herein I_0, indicated the fluorescence intensity of free RND membrane; I indicated the fluorescence intensity upon adding Hg^{2+}) of fluorescence intensity at ca. 590 nm was observed. The color of the membrane also changed from colorless to pink-red. This phenomenon elicited that the spectral response of compound RND to Hg^{2+} was probably due to the Hg^{2+}-induced ring-opening of rhodamine spirolactam [50]. Furthermore, the fluorescence intensities of the optode membrane gradually increased with increasing Hg^{2+} concentrations, which constituted the quantitative basis of the proposed optical sensing sensor. In contrast, addition of other metal cations showed a little difference in fluorescence intensity and scarcely color changes. According to the aforementioned literatures [46–48], a proposed binding mode of Hg^{2+} with RND was shown in Scheme S2.

Under the optimized conditions, the linearity was determined by plotting the fluorescence enhancement value ΔI ($\Delta I = I - I_0$, where I_0 and I were the fluorescence emission intensities before and after Hg^{2+} was added, respectively) against the logarithm of Hg^{2+} concentration, obtaining a linear equation of $\Delta I = 114.61 \log C + 1170.00$ ($R = 0.9932$) in the concentration range of 1.0×10^{-9}–2.0×10^{-3} M Hg^{2+}. Response curve was illustrated in the inset of Fig. 3. The optical response was nonlinear relationship between ΔI and $\log C_{Hg^{2+}}$ when Hg^{2+} concentration was lower than 1.0×10^{-9} M (data not shown). The limit of detection (LOD) based on 3σ of the blank was 8.1×10^{-10} M, which was

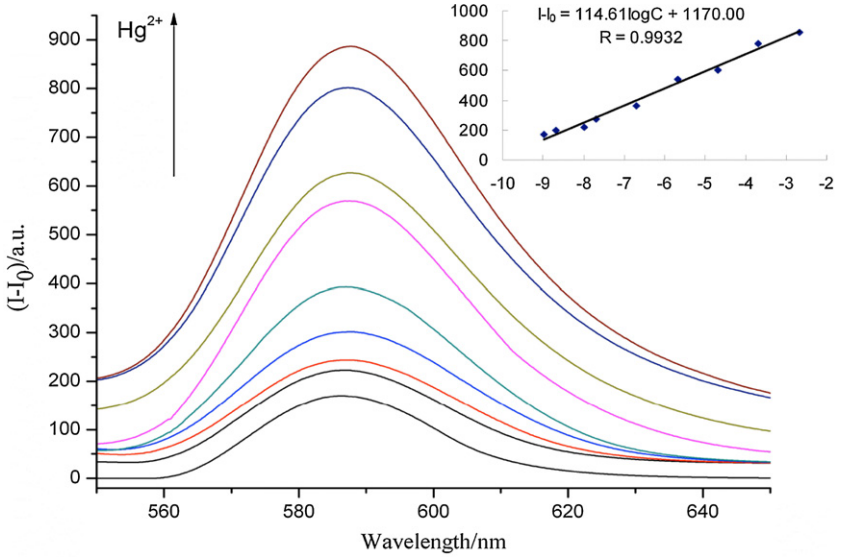

Fig. 3. Fluorescence response of RND sensing membrane upon addition of Hg^{2+} in Tris–HCl buffer solution at pH 6.5 (excitation at 374 nm), where, I_0 and I represent the fluorescence intensity at ca. 590 nm in the absence and presence of Hg^{2+}, respectively. Inset: plot of ΔI as a function of the log $C_{Hg^{2+}}$.

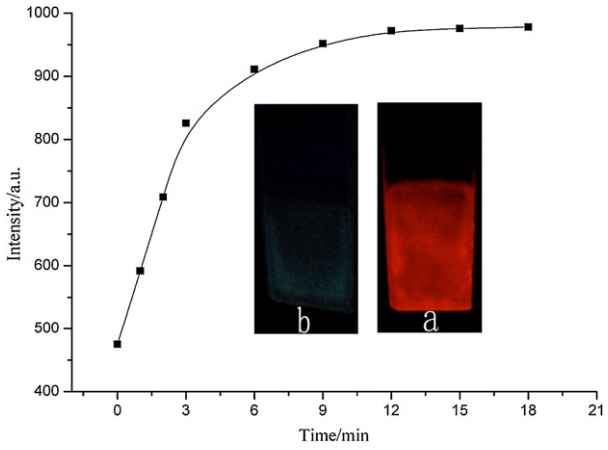

Fig. 4. Typical response curve of the proposed optode at the excitation wavelength of 374 nm as a function of time when the optode was exposed to 2.0 μM Hg^{2+}. Inset: the photographs of the proposed sensor under UV light (a. with Hg^{2+}; b. without Hg^{2+}).

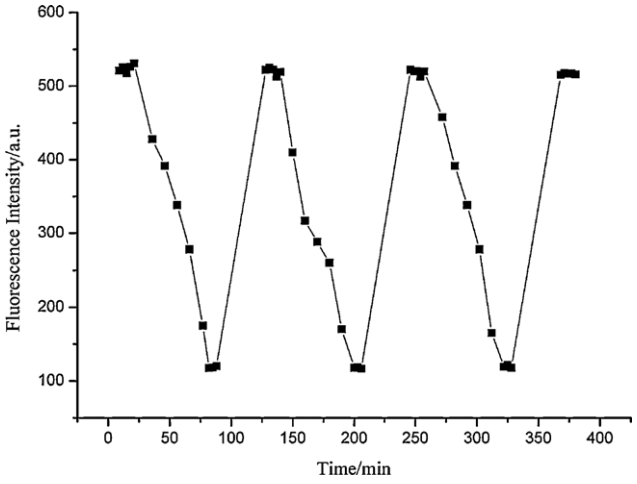

Fig. 5. Variation of the fluorescence intensity of the optode membrane at 374 nm for repetitively exposing into 2.0 μM of mercuric ion and a mixture solution of 0.01 M NaOH and 0.1 M NaCl.

much lower than the maximum contamination level defined by the U.S. EPA [51] (1×10^{-8} M) in domestic water supplies. The present method was compared to the analytical methods previously published in literatures [8,24,26,27,44,45,52–55] using PVC membrane for mercuric ion analysis in terms of the detection limits (LODs) and measured signals (Table 5). As can be seen, most of the reported sensors [8,24,26,27,52–55] were based on fluorescence quenching, reflectance or absorbance measurement, only a few of them [44,45] were based on fluorescence enhancement mechanism. However, the LOD of this sensor was at least one order of magnitude lower than that of the reported method [45], showing high sensitivity of the proposed sensor. The resulting cocktails [44] were spread onto a polyester support (Mylar TM type) instead of a glass slide, which would cause relatively poor performance in terms of alkali resistance.

The response time ($t_{95\%}$) of the optode depended on the membrane thickness and the concentration of Hg^{2+}. The thickness, alternatively, depends on the volume of the coating solution applied to the substrate and the area over which it spreads. The membrane thickness was estimated to be ~10 μm when a 35 μl aliquot of the coating solution (containing 100 mg membrane ingredients in 2 ml THF) was applied over an area of ~1.92 cm^2. According to the experiment, when the thickness of the membrane reached the order of micrometers, no apparent difference of response time can be observed. On the other hand, the time required for the optode to reach a stable response increased when the concentration of Hg^{2+} was decreased. In the entire concentration range from 2.0×10^{-3} to 1.0×10^{-9} M Hg^{2+}, the membrane response was found to reach stable in about 3–15 min (Fig. 4).

3.4. Regeneration and reproducibility

After contacting with Hg^{2+} solution, the sensor membrane must be regenerated by using a suitable stripping reagent. Preliminary experiments have been performed to select a suitable regenerating solution. Thiourea, which was examined at several concentrations, was not a suitable regenerating solution, because it can irreversibly compete with RND for Hg^{2+} ions. Anions such as I^-, Br^-, S^{2-}, SCN^-, and EDTA (sodium salt) were inefficient to perform this task. Other several compounds including L-cysteine, DL-homocysteine, thioacetamide, histidine, thiamine hydrochloride were not useful for this study. The best result was obtained by applying a mixture solution containing sodium hydroxide and sodium chloride, which gave membrane regeneration time of 60 min. By increasing the NaOH concentration up to 0.01 M, the stripping process for the sensor in contact with different Hg^{2+} concentrations was accelerated. Higher concentrations of this reagent not only did not produce any additional improvement but also forbade the response of the sensor. Therefore, 0.01 M NaOH (containing 0.1 M NaCl) was used as regeneration solution.

The reproducibility and reversibility of the optode were assessed by alternatively recording the fluorescence intensity for blank buffer solution and 2.0 μM Hg^{2+}. After each measurement, the regeneration and conditioning steps were carried out. We consecutively performed eight determinations to the same membrane sensor, with R.S.D.s of 4.7% (Fig. 5). The between membrane reproducibility was also obtained by preparing six membranes using the same membrane solution. The resulting coefficient of variation for these membranes was found to be 8.5%, and test results showed no significant difference between the produced membranes.

3.5. Stability

To study the stability of the sensor, its response in contact with 2.0 μM Hg^{2+} in Tris–HCl buffer of pH 6.5 was recorded over a period of 2 h with an interval of 5 min. The mean fluorescence intensity value and relative standard deviation was found to be 910 and 8.8%, respectively ($n = 25$). This shows the optode membrane has good short-term stability. Additionally, during the continuous measurements of the membrane of 7 days (dark storage), the fluorescence

Table 2
Effect of different interferents on the fluorescence signals of the optode membrane.[a]

Interference	Concentration (M)	Relative error (%)
Pb^{2+}	5.00×10^{-3}	3.92
Sr^{2+}	5.00×10^{-3}	5.00
Cd^{2+}	3.80×10^{-3}	−1.40
Al^{3+}	3.50×10^{-3}	3.53
Na^{+}	2.90×10^{-3}	4.90
Mg^{2+}	1.40×10^{-3}	4.06
Mn^{2+}	2.10×10^{-4}	2.39
Ni^{2+}	6.30×10^{-4}	−2.20
Fe^{3+}	6.60×10^{-4}	−2.77
Ca^{2+}	6.00×10^{-4}	3.15
Cu^{2+}	6.00×10^{-4}	3.72
Co^{2+}	3.00×10^{-4}	3.79
Ba^{2+}	6.00×10^{-5}	4.91
K^{+}	1.20×10^{-5}	3.70
Zn^{2+}	1.20×10^{-5}	3.38

[a] The concentration of Hg^{2+} was fixed at 2.0 μM (pH 6.5).

Table 3
Results of Hg^{2+} determination in samples.

Sample	Hg^{2+} spiked (M)	Sensor method		ICP	
		Hg^{2+} recovered (M)[a]	Recovery (%)	Hg^{2+} recovered (M)[a]	Recovery (%)
Pond water	–	<Limit of detection	–	<Limit of detection	–
	4.00×10^{-6}	$(4.11\pm0.24)\times10^{-6}$	102.8	$(4.15\pm0.20)\times10^{-6}$	103.8
	2.00×10^{-5}	$(2.10\pm0.17)\times10^{-5}$	105.0	$(1.91\pm0.11)\times10^{-5}$	95.5
	3.90×10^{-5}	$(3.84\pm0.38)\times10^{-5}$	98.5	$(3.87\pm0.22)\times10^{-5}$	99.2
	4.00×10^{-7}	$(4.17\pm0.35)\times10^{-7}$	104.2	$(3.88\pm0.12)\times10^{-7}$	97.0
Fish	–	<Limit of detection	–	<Limit of detection	–
	4.00×10^{-6}	$(3.88\pm0.31)\times10^{-6}$	97.0	$(4.13\pm0.21)\times10^{-6}$	103.2
	2.00×10^{-5}	$(1.92\pm0.26)\times10^{-5}$	96.0	$(2.08\pm0.13)\times10^{-5}$	104.0
	3.90×10^{-5}	$(4.05\pm0.47)\times10^{-5}$	103.8	$(4.01\pm0.23)\times10^{-5}$	102.8
	4.00×10^{-7}	$(4.14\pm0.25)\times10^{-7}$	103.5	$(4.19\pm0.17)\times10^{-7}$	104.8

[a] Average value of three determinations.

intensity of the optode membrane dropped by about 10.6% (the results were compared with those of the first day). The reason for less stability of the membrane in ambient condition might be owing to the photodecomposition and/or leaching of the membrane components. Apparently, the lifetime of the proposed sensor was acceptable for continuous analytical applications.

3.6. Selectivity

To evaluate the metal ion bonding property and selectivity, the competition experiments were also conducted by adding the interfering ions to solutions containing 2.0 μM Hg^{2+} in Tris–HCl buffer, pH 6.5. The maximum foreign ion/Hg^{2+} concentration ratio assayed was 2.5×10^{3}. The resulting relative error is defined as RE (%) = $[(F_1 - F_0)/F_0]\times100$ (herein, F_0 was the fluorescence intensity of Hg^{2+} solution, while F_1 was the fluorescence intensity after adding the interfering ion into Hg^{2+} solution). As seen from Table 2, relative error is less than ±5%, which is recognized as tolerated. Among these ions Sr^{2+}, Na^{+}, K^{+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Fe^{3+}, Ba^{2+}, Al^{3+}, Hg^{2+} and Pb^{2+}, only Hg^{2+} can trigger both a fluorescence enhancement and a pink-red color change, which rendered its application feasible for the analysis of real samples. Maximum interference was observed from Ba^{2+}, K^{+} and Zn^{2+} ions. There were several ways that could be used to eliminate or minimize the interference of these cations in different samples, including separation, masking agents and regression models.

3.7. Applications

Finally, the proposed sensor was applied to the determination of Hg^{2+} in fish and environmental water samples. As no distinct signal of Hg^{2+} was observed, all samples were spiked with Hg^{2+} standard solutions and then analyzed with the proposed sensor. The results were compared with the data obtained from inductively coupled plasma (Optima 2100 DV, Pekin-Elmer, USA) spectrometric measurements (Table 3). From the data given in Table 3, it is readily seen that the mercury contents of different samples determined by the proposed Hg^{2+}-sensor are in satisfactory agreement with those obtained by ICP-OES measurements. One can see that the recovery study exhibited satisfactory results.

Table 4
Analytical parameters of the optimized optode membrane used for the determination of mercuric ion.

Parameter	Values or range
Reproducibility (as R.S.D.%)	Bulk method: within membrane, 4.7% for 2.0 μM Hg^{2+} ($n=8$); Bulk method: between membrane, 8.5% for 2.0 μM Hg^{2+} ($n=6$) Flow method: within membrane, 2.3% for 2.0 μM Hg^{2+} ($n=4$); Flow method: between membrane, 5.2% for 2.0 μM Hg^{2+} ($n=4$)
Stability	Short term (2 h): 8.8% R.S.D. with 2.0 μM Hg^{2+} ($n=25$) Long term (life time): ~7 days in ambient condition
Response range	Bulk method: 1.0×10^{-9}–2.0×10^{-3} M Hg^{2+} Flow method: 2.0×10^{-10}–2.0×10^{-3} M Hg^{2+}
Detection limit	Bulk method: 8.1×10^{-10} M Flow method: 8.3×10^{-11} M

3.8. Flow-through measurement

The flow-through cell was placed in the spectrofluorometer sample holder. Similar to the measurements carried out in the bulk method, each measurement cycle in the flow method also included three steps. The carrier solution contained pH 6.5 Tris–HCl buffer was pumped for 25 min through the sensing membrane at a constant flow rate of 0.2 ml min^{-1}. Once a stable baseline was obtained (I_0 signal), 5.0 ml (25 min) of Hg^{2+} solution was pumped through the system and the increase of the fluorescence signal was evaluated (I signal). Finally, the sensing membrane was regenerated with 0.01 M NaOH (containing 0.1 M NaCl) for an additional 60 min, and

Table 5
Some reported sensors using PVC membrane for the determination of Hg^{2+}.

Reagent	Working range (M)	LODs[a] (M)	Measured signal	Reference
L	1.0×10^{-4}–5.0×10^{-12}	8.0×10^{-13}	Fluorescence	[8]
EHT	5.0×10^{-10}–5.0×10^{-5}	1.8×10^{-10}	Fluorescence	[44]
MTZ	2.0×10^{-10}–1.5×10^{-5}	5.0×10^{-11}	Absorbance	[27]
PAN	1.0×10^{-5}–2.0×10^{-4}	5.5×10^{-7}	Reflectance	[24]
H2tpp	2.26×10^{-7}–4.52×10^{-5}	4.0×10^{-8}	Fluorescence	[26]
Tetraarylborate/porphyrin	1.0×10^{-7}–2.6×10^{-6}	1.0×10^{-7}	Fluorescence	[45]
LX	2.95×10^{-10}–3.20×10^{-3}	1.01×10^{-10}	Absorbance	[52]
DTPP	5.2×10^{-7}–3.1×10^{-4}	5.2×10^{-7}	Fluorescence	[53]
2-MP	2.0×10^{-9}–2.0×10^{-5}	4.0×10^{-10}	Absorbance	[54]
H_3(tpfc)	1.2×10^{-7}–1.0×10^{-4}	1.2×10^{-7}	Fluorescence	[55]
RND	1.0×10^{-9}–2.0×10^{-3}	8.1×10^{-10}	Fluorescence	This work

[a] LODs is limit of detection (M).

buffer solution was pumped through the flow cell until the fluorescence intensity of the optode reached the original blank value.

A flow rate of 0.2 ml min^{-1} for all the solutions was selected as a compromise between sensitivity and sampling frequency. The corresponding calibration plot of ΔI ($\Delta I = I - I_0$) versus $\log C_{Hg^{2+}}$ exhibited a useful response range from 2.0×10^{-10} to 2.0×10^{-3} M. The regression equation was $\Delta I = 37.30 \log C + 579.65$, when the concentration of Hg^{2+} was expressed in mol L^{-1}, with a correlation coefficient of 0.9983. The LOD, as defined in Section 3.3, was 8.3×10^{-11} M Hg^{2+} (Fig. S2).

The repeatability was evaluated by performing four measurements on 2.0 μM Hg^{2+}. The R.S.D. value was 2.3%. The between membrane reproducibility was evaluated by preparing four membranes with the same membrane solution and mounting them in the flow-through cell as described in Section 2. The R.S.D. value in this case was 5.2%. The results collected in Table 4 demonstrated lower detection limit, greater response range and the advantage of continuous analyses of samples in the flow system compared to the bulk assay.

4. Conclusions

An optical sensor based on immobilization of RND into PVC membrane was developed and used for the determination of Hg^{2+} in bulk and flow systems. On the basis of the results discussed in this letter, the proposed rhodamine-based optode membrane showed good optical and mechanical properties, wide response range (7 orders of magnitude for bulk method; 8 orders of magnitude for flow method), acceptably response time and extremely high selectivity to Hg^{2+} over other competing metal ions. The sensor has been successfully employed for the determination of trace mercury in water and fish samples. The LODs, 8.1×10^{-10} M in the bulk and 8.3×10^{-11} M in the flow systems were reached. The very low detection limit together with the membrane's high selectivity over several other cations, make the proposed optode potential useful for determining this ion in other materials. All the reagents needed were sealed in the sensing membrane, and thus the detection of Hg^{2+} here only required buffering the sample. The proposed fluorescence optode was useful for routine analysis with portable equipment and the analysis in situ without prior sampling manipulation and treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2012.01.013.

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