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A sensitive and selective fluorescent sensor for the determination of mercury(II) based on a novel triazine-thione derivative

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ABSTRACT

A sensor membrane based on the fluorescence enhancement of a novel triazine-thione derivative, 4-ethyl-5-hydroxy-5,6-di-pyridin-2-yl-4,5-dihydro-2H-[1,2,4]triazine-3-thione, was capable of determining mercury(II) with high selectivity over the range 5.0×10^{-10} and 5.0×10^{-5} mol L^{-1} with a limit of detection of 1.8×10^{-10} mol L^{-1} (0.036 μg L^{-1}). The sensor can be regenerated using 5% thiourea in 1.0 mol L^{-1} HCl solution. The sensor also displayed unique selectivity toward mercury(II) ion with respect to other common metal cations and was applied to the determination of mercury(II) in tap water and human hair samples. The accuracy of the results were comparable to those obtained by cold vapour atomic fluorescence spectrometry.

1. Introduction

Mercury is a potentially toxic environmental pollutant that is among the most highly bioconcentrated trace metals in the human food chain and several international committees have targeted mercury for special attention with regard to its emissions and effects on human health [1,2]. Hence, determination of trace mercury is important for regulatory and control purposes. The most widely used methods for determining mercury are cold vapour atomic absorption spectrometry [3], cold vapour atomic fluorescence spectrometry [4], X-ray fluorescence spectrometry [5], inductively coupled plasma-mass spectrometry [6] and voltammetry [7]. With regard to sensitivity and accuracy, whilst these methods are efficient tools for mercury determination, they are time-consuming, expensive and require sophisticated equipment [8]. Thus, much interest has attended the development of fluorescent sensors that offer distinct advantages in terms of sensitivity, selectivity, response time and remote sensing [9,10].

A variety of reagents has been used for constructing mercury ion optical sensors, these including 1-(2-pyridylazo)-2-naphthol (PAN) [11], 1,5-di-(2-fluorophenyl)-3-mercaptoformazan (F_2H_2Dz) immobilized and plasticized with tri-*n*-butylphosphate (TBP) polyurethane foam (PUF) [12], *p*-dimethylaminobenzaldehyde thiosemicarbazone (DMABTS) [13], 5,10,15,20-tetraphenylporphyrin (H2tpp) [14], 4-(2-pyridylazo)resorcinol (PAR) [15], reflectance spectroscopy based on zinc-dithizone on XAD-7 [16] and 4-phenolazo-3-aminorhodanine into polyacrylonitrile fibers filled into ion exchangers [17].

Generally, sol-gel glasses [18,19] or polymer matrices are used for the preparation of optical chemical sensors. Polyvinlyl chloride (PVC) has been used for the preparation of the membrane sensors due to its relatively low cost, good mechanical properties and amenability to plasticization [20]. Shamsipur et al. [21] prepared a Hg^{2+} fluorescence sensor by incorporating 1-(dansylamidopropyl)-1-aza-4,10-dithia-7-oxacyclododecane (L) as a neutral Hg^{2+}-selective fluoroionophore in a plasticized PVC membrane containing potassium tetrakis(*p*-chlorophenyl)-borate as a lipophilic anionic additive. A selective optical sensor membrane for the detection of mercury(II) was proposed by Murkovic and Wolfbeis [22] in which the sensing layer comprised plasticized PVC containing a lipophilic borate salt as a reagent for Hg(II) and an amphiphilic oxacarbocyanine dye as optical transducer. He et al. [23] developed a fluorescent sensor for Hg^{2+} using 5,10,15-tris (pentafluorophenyl)corrole H_3(tpfc) as fluorophore which

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displayed linear response towards Hg^{2+} in the concentration range 1.2×10^{-7} mol L^{-1} to 1.0×10^{-4} mol L^{-1}. Recently Yang et al. [24] used tetra(*p*-dimethylaminophenyl)porphrin (TDMAPP) as sensing reagent, which could be applied to the quantification of Hg^{2+} within the linear range of 4.0×10^{-8} mol L^{-1} to 4.0×10^{-6} mol L^{-1}. Cano-Raya et al. [25,26] and Kuswandi et al. [27] have reported disposable sensors for Hg^{2+} based on plasticized PVC membrane that function in the working ranges and/or limits of detection (LOD) of mercury sensors have been summarized in Table 1.

This paper concerns the photocharacterisation of the novel, fluorescent dye, 4-ethyl-5-hydroxy-5,6-di-pyridin-2-yl-4,5-dihydro-2H-[1,2,4]triazine-3-thione (EHT) and its use as a selective, sensitive optical sensor for mercury ions. The sensing procedure developed has been successfully employed for the determination of trace mercury in water and hair samples.

2. Experimental

2.1. Reagents

The polymer membrane components, polyvinylchloride (PVC) (high molecular weight) and the plasticizers, bis-(2-ethylhexyl) phtalate (DOP), bis(2-ethylhexyl)sebecate (DOS), bis-(2-ethylhexyl) adipate (DAO) and 2-nitrophenyl octyl ether (NPOE) were obtained from Fluka. The lipophilic anionic additive reagent potassium tetrakis-(4-chlorophenyl) borate (PTCPB) was supplied by Aldrich. Absolute ethanol (EtOH), tetrahydrofuran (THF), dichloromethane (DCM), acetone, nitric acid, hydrogen peroxide and anthracene were purchased from Merck. Thiourea was obtained from BDH. Sheets of Mylar-type polyester (Dupont, Switzerland) were used as support. All solutions were prepared with glass-distilled water.

The synthesis of EHT (Fig. 1) was undertaken according to the published procedure [28].

2.2. Instrumentation

UV–Vis absorption spectra were recorded using Varian Cary 100 bio UV–Visible spectrophotometer. All fluorescence measurements were carried out on a Shimadzu RF-5301 PC spectrofluorimeter with a Xenon short arc lamp as the light source. PSA 10.004 atomic fluorescence spectrometer was also used for mercury measurements. Measurement of pH was performed using a WTW 82362 Weliheim pH 330i pH-meter calibrated with Merck pH standards of pH 4.00, 7.00 and 10.00. The film thicknesses of the sensing slides were measured with Ambios Technology XP-1 HGH Resolution surface profiler.

2.3. Structural characterization of the 4-ethyl-5-hydroxy-5,6-di-pyridin-2-yl-4,5-dihydro-2H-[1,2,4]triazine-3-thione

The novel 1,2,4-triazine-3-thione derivatives were obtained using a one-step process [28]. The structure of the compounds were determined using infrared (IR), ^{1}H NMR, and ^{13}C NMR spectroscopic methods as well as elemental analysis.

Yield: 1.75 g, 56%; mp 219 °C; FT-IR (KBr, cm^{-1}): 3175 (–OH), 3134 (–NH–), 2986–2928 (aliphatics), 1519 (thioamide I), 1243 (thioamide II), 1079 (thioamide III), 634 (thioamide IV) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.72 (t, J = 7.0 Hz, 3H, –CH3), 3.54 (sextet, 1H, J = 6.6 Hz, $-CH_2A-$), 3.69 (sextet, 1H, J = 6.7 Hz, $-CH_2B-$) (bothare centered), 7.15–7.18 (ddd, 1H, J_1 = 7.4 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz), 7.23–7.27 (ddd, 1H, J_1 = 6.7 Hz, J_2 = 4.7 Hz, J_3 = 1.1 Hz), 7.68–7.97 (m, 4H, aromatics), 8.04 (s, 1H, –NH–, D_2O exchangeable), 8.17–8.19 (ddd, 1H, J_1 = could not be detected, J_2 = 4.8 Hz, J_3 = ~1 Hz), 8.37–8.40 (ddd, 1H, J_1 = could not be detected, J_2 = 4.4 Hz, J_3 = ~1 Hz), 11.92 (s, 1H, –OH, D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ 170.46, 160.93, 153.50, 149.15, 148.33, 143.73, 137.35, 137.15, 123.98, 123.81, 122.30, 122.04, 82.94, 41.64, 14.25. Anal. calcd. for $C_{15}H_{15}N_5OS$: C, 57.49; H, 4.82; N, 22.35; S, 10.23. Found: C, 57.65; H, 5.03; N, 22.71; S, 9.96.

2.4. Preparation of polymer film

The membrane cocktail was prepared by dissolving a mixture of 120 mg of PVC, 240 mg of plasticizer (DOP), 2.0 mg of PTCPB and 2.0 mg of EHT dye in 1.5 mL of dried THF. The prepared mixtures contained 33% PVC and 66% plasticizer by weight which is in accordance with literature [29,30]. The resulting cocktails were spread onto a polyester support (Mylar TM type) located in a THF-saturated desiccator. The polymer support is optically fully transparent, ion impermeable and exhibits good adhesion to PVC. The films were kept in a desiccator in the dark. This way the photostability of the membrane was ensured and the damage from the ambient air of the laboratory was avoided. Each sensor film was cut to a size of 13 × 50 mm. The film thicknesses of the sensing slides

Table 1
Some reported optical sensors for the determination of Hg^{2+}.

Reagent	Working range (mol L^{-1})	Limit of detection (mol L^{-1})	Measured signal	Reference
PAN	1.0×10^{-5} to 1.0×10^{-3}	5.5×10^{-7}	Reflectance	[11]
F_2H_2Dz	NR[b]	2.5×10^{-7}	Absorbance	[12]
DMABTS	0.0 to 5.77×10^{-6}	7.7×10^{-7}	Fluorescence	[13]
H2tpp	2.26×10^{-7} to 4.52×10^{-5}	4.0×10^{-8}	Fluorescence	[14]
PAR	1.1×10^{-6} to 6.6×10^{-6}	5.5×10^{-7}	Absorbance	[15]
zinc-dithizone	0.0 to 8.9×10^{-3}	2.5×10^{-7}	Reflectance	[16]
H2tpp	5.0×10^{-6} to 1.0×10^{-4}	3.6×10^{-6}	Fluorescence	[18]
2-(5-amino-3,4-dicyano-2H-pyrrol-2-ylidene)-1.1.2-tricyanoethanide	5.0×10^{-4} to 5.0×10^{-3}	5.0×10^{-5}	Absorbance	[19]
L	5.0×10^{-12} to 1.0×10^{-4}	8.0×10^{-13}	Fluorescence	[21]
Tetraphenylborate/oxacarbocyanine	2.0×10^{-7} to 3.2×10^{-6}	1.0×10^{-7}	Fluorescence	[22]
H_3(tpfc)	1.2×10^{-7} to 1.0×10^{-4}	NR[b]	Fluorescence	[23]
TDMAPP	4.0×10^{-8} to 4.0×10^{-6}	8.0×10^{-9}	Fluorescence	[24]
1,4,7,10-tetraazacyclododecane	3.0×10^{-7} to 5.1×10^{-6}	3.0×10^{-7}	Absorbance	[25]
Tetraarylborate/porphyrin	1.0×10^{-7} to 2.6×10^{-6}	1.0×10^{-7}	Fluorescence	[26]
Trityl-picolinamide	5.0×10^{-7} to 5.0×10^{-4}	5.0×10^{-7}	Absorbance	[27]
2-mercapto-2-thiazoline	2.0×10^{-10} to 1.5×10^{-5}	5.0×10^{-11}	Absorbance	[34]
Hexathiacyclooctadecane	2.1×10^{-7} to 1.2×10^{-4}	2.0×10^{-7}	Absorbance	[35]
EHT[a]	5.0×10^{-10} to 5.0×10^{-5}	1.8×10^{-10}	Fluorescence	–

[a] Proposed sensor
[b] NR: not reported

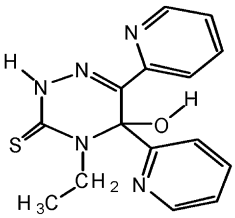

Fig. 1. Structure of 4-ethyl-5-hydroxy-5,6-di-pyridin-2-yl-4,5-dihydro-2H-[1,2,4] triazine-3-thione (EHT) dye.

were measured with Ambios Technology XP-1 HGH Resolution surface profiler and found to be 4.84 ± 0.052 μm for PVC matrices ($n = 8$).

Absorption and fluorescence emission spectra of PVC membranes were recorded in quartz cells which were filled with sample solution. The polymer films were placed in diagonal position in the quartz cell. The advantage of this kind of placement was to improve the reproducibility of the measurements. All of the experiments were operated at room temperature, 25 ± 1 °C. The membranes were not conditioned before use.

3. Results and discussion

3.1. Spectral characterization studies

The emission and excitation spectra of EHT dye were recorded in the solvents of different polarities and PVC matrix. The gathered excitation-emission spectra of the EHT dye are shown in Fig. 2. The Stokes shift values, $\Delta\lambda_{ST}$ (the difference between excitation and emission maxima) were extracted from spectral data which are given in Table 2. Since larger Stokes shifts are obtained in polar solvents [9], the highest Stokes shift for the EHT dye was observed in EtOH in this study. The EHT dye exhibited higher fluorescence intensity in PVC matrix compared to that in the solvents. The immobilization of dye molecules in solid matrix may reduce intramolecular motions and rearrangements, thus leading to enhanced fluorescence capability.

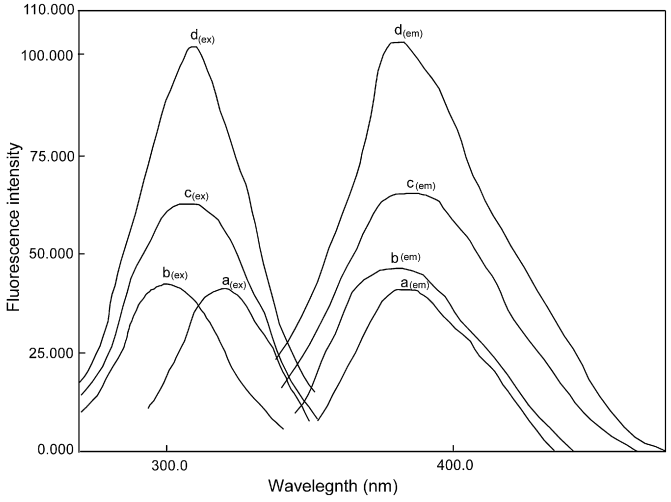

Fig. 2. Emission and excitation spectra of EHT dye in different solvents and PVC. (a) DCM ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 388$ nm), (b) EtOH ($\lambda_{ex} = 300$ nm, $\lambda_{em} = 383$ nm), (c) THF ($\lambda_{ex} = 318$ nm, $\lambda_{em} = 390$ nm), (d) PVC ($\lambda_{ex} = 320$ nm, $\lambda_{em} = 387$ nm).

Table 2
Emission and excitation spectra related data of EHT and EHT–Hg^{2+} complex.

Solvent	Excitation wavelength λ_{ex} (nm)	Emission wavelength λ_{em} (nm)	Stokes shift $\Delta\lambda_{ST}$ (nm)	Refractive index n	Quantum yield Φ_F
DCM	330	388	58	1.4241	0.024
THF	318	390	72	1.4070	0.017
EtOH	300	383	83	1.3614	0.012
PVC	320	387	67	1.5247	0.035
PVC (Complex)	320	387	67	1.5247	0.068

3.2. Fluorescence quantum yield calculations

Fluorescence quantum yield values (Φ_F) of the EHT compound were calculated employing the comparative William's method which involves the use of well-characterized standards with known (Φ_F) values [31]. Anthracene ($\theta_{ST} = 0.27$) in ethanol was used as a standard [32]. For this purpose, the UV–vis absorbtion and emission spectra of six different concentrations of reference standard and EHT were recorded. The integrated fluorescence intensities were plotted versus absorbance for the reference standard and the dye. The gradients of the plots are proportional to the quantity of the quantum yield of the studied molecules. The data obtained and quantum yield (Φ_F) values calculated according to Eq. (1) are shown in Table 2.

$$\theta_x = \theta_{ST}\left(\frac{Grad_x}{Grad_{ST}}\right)\left(\frac{n_x^2}{n_{ST}^2}\right) \quad (1)$$

where ST and x denote standard and sample, respectively, Grad is the gradient from the plot and n is the refractive index of the solvent or polymer matrix material. As seen from the data in Table 2, the quantum yield of the EHT dye depends on the polarity of organic solvents. Upon increasing the solvent polarity, the quantum yields found decreased significantly, that is in accordance with the literature [9].

3.3. Response of sensing membrane to Hg^{2+}

Preliminary experiments showed that EHT immobilized into PVC membrane incorporating plasticizer and PTCPB has the necessary conditions of a suitable ligand for detection of Hg^{2+}. In the presence of mercury ion, a relatively strong complex is formed between Hg^{2+} and EHT with a corresponding increase in the quantum yield (Table 2) and thus the fluorescence intensity.

In order to determine the stoichiometry of the EHT-Hg^{2+} complex, the method of continuous variations (Job's method) was used (Fig. 3). In Job's method different amounts of stock solutions of metal and ligand are mixed varying the mole ratio of reactants. The result obtained from the Job plot indicates the formation of a 1:1 complex between EHT and Hg^{2+}. The association constant (K_a) of EHT to Hg^{2+} according to the 1:1 binding model by non-linear fitting of the spectrometric titration curve [33] is obtained as 1.76×10^5 L mol^{-1} (Fig. 4).

Thiol group forms as a result of tautomerism in the ligand [28]. Since the complex has 1:1 stoichiometry the complex is expected to be formed between the more acidic –SH group of the ligand and Hg^{2+}.

Fig. 5 shows the fluorescence emission spectra of the sensing membrane exposed to the solutions containing different concentrations of Hg^{2+} by exciting at 320 nm. Noticeable increase of the fluorescence emission intensity appeared in the presence of Hg^{2+}, which was attributed to the formation of a complex between EHT

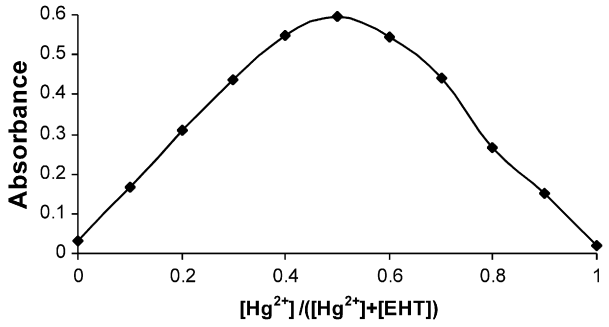

Fig. 3. Job's plot for complex formation of EHT with Hg^{2+} in THF (λ_{abs} = 320 nm, [EHT] + [Hg^{2+}] = 1.0 × 10^{-6} mol L^{-1}).

dye and Hg^{2+}, and the fluorescence intensities of the sensing membrane gradually increased with increasing Hg^{2+} concentrations, which have been utilized as the quantitative basis of the Hg^{2+} sensor.

Using the optimized conditions, the linearity was determined by plotting the relative fluorescence enhancement value $\Delta F/F_0$ ($\Delta F = F - F_0$, where F_0 and F are the fluorescence emission intensities before and after Hg^{2+} was added, respectively) against Hg^{2+} concentration, obtaining a linear equation of $y = 0.3271x + 3.3896$ (R^2 = 0.9973) in the concentration range of 5.0 × 10^{-10} to 5.0 × 10^{-5} mol L^{-1} Hg^{2+}.

3.4. Effect of membrane composition

The membrane composition is well documented to largely influence the response characteristics and working concentration range of the optical sensors [14,19,21,25–27,34,35]. Several solvent mediators such as DOP, DOS, DAO, and NPOE were tested as potential plasticizers for preparing the membrane. The membranes were prepared from a mixture of 120 mg PVC, 240 mg of the plasticizer, 2.0 mg of PTCPB and 2.0 mg of EHT dye. Those compounds were dissolved in 1.5 mL THF as described in the procedure above. The fluorescence measurements were made for different concentration of Hg(II) ions for the membranes with different types of the plasticizers. The results are shown in Table 3. We found that DOP acts superior with respect to other common plasticizers used in the construction of the Hg^{2+} optical sensor, because of the membrane containing DOP revealed best physical properties with maximum sensitivity and minimum leaching, probably due to highest lipophilicity and suitable polarity [36].

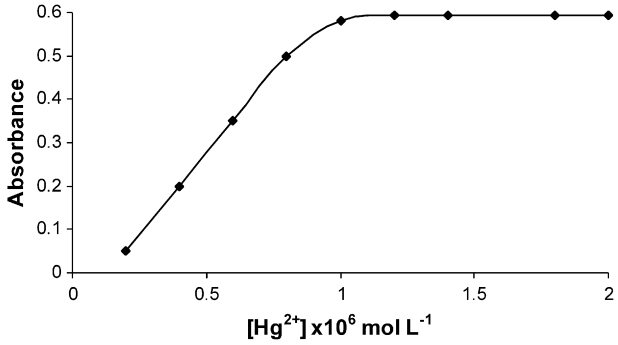

Fig. 4. Non-linear fitting of the spectrometric titration curve of EHT with Hg^{2+} in THF (λ_{abs} = 320 nm, [EHT] = 1.0 × 10^{-6} mol L^{-1}).

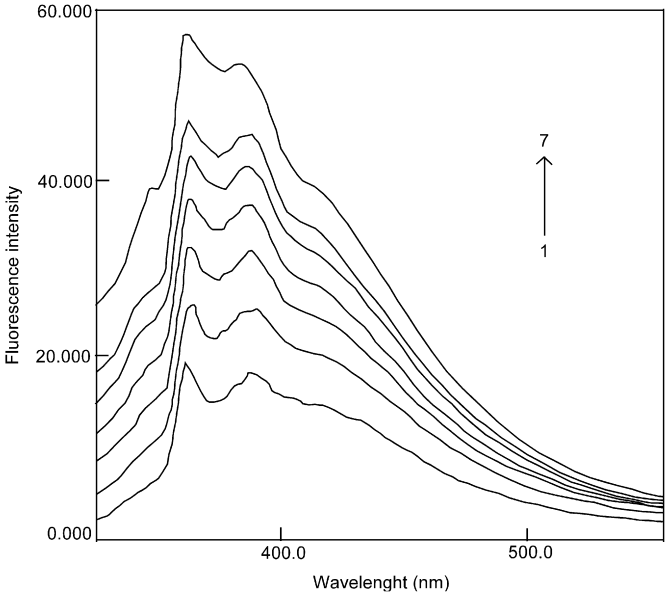

Fig. 5. The fluorescence emission spectra of the PVC sensing membrane exposed to the solutions containing different concentrations of Hg^{2+} at pH 5.5: (1) 0; (2) 5.0 × 10^{-10} mol L^{-1}; (3) 5.0 × 10^{-9} mol L^{-1}; (4) 5.0 × 10^{-8} mol L^{-1}; (5) 5.0 × 10^{-7} mol L^{-1}; (6) 5.0 × 10^{-6} mol L^{-1}; (7) 5.0 × 10^{-5} mol L^{-1} (λ_{ex} = 320 nm).

3.5. Effect of pH value

The complexation reaction of the optode with Hg^{2+} ion is affected by the pH of the solution. Fig. 6 shows the effect of the pH on the response of the optode membrane. The fluorescence measurements were made for 5.0 × 10^{-6} mol L^{-1} Hg^{2+} ion at different pH values. It can be seen that, in the section of lower pH values, the fluorescence intensity of the optode decreased with decreasing pH value. This occurrence might be caused by extraction of H^+ from aqueous solution into the optode membrane at high acidity, which caused the protonation of the nitrogen atom on EHT and decreased the mobility of its π-electrons. On the other hand, the reduced optical response of the proposed sensor at pH >10.0 could be due to a possible slight swelling of the polymeric membrane under alkaline conditions and the partial precipitation of Hg(II) as $Hg(OH)_2$. From Fig. 6 one can see that in a range of pH from 5.0 to 8.0, acidity does not affect the determination of Hg^{2+} with the proposed optode. This result simplifies the practical application of the sensor in the determination of Hg^{2+} concentration in real samples. Therefore, pH 5.5 CH_3COOH/CH_3COO^- (0.1 mol L^{-1}) buffer solution was selected as optimal experimental condition.

3.6. Regeneration of the optode

After the contact of the optode membrane with Hg^{2+} solution, it must be regenerated using a suitable stripping reagent. Preliminary experiments were performed to select a suitable regenerating

Table 3
Effect of different type of plasticizer on the response of the sensor for determination of Hg(II) at pH 5.5.

Plasticizer	Working concentration range (mol L^{-1})	Response time (s) (5.0 × 10^{-6} mol L^{-1} Hg(II))
DOP	5.0 × 10^{-10} to 5.0 × 10^{-5}	180
DOS	5.0 × 10^{-10} to 1.0 × 10^{-6}	220
DOA	5.0 × 10^{-9} to 1.0 × 10^{-6}	210
NPOE	1.0 × 10^{-8} to 5.0 × 10^{-5}	195

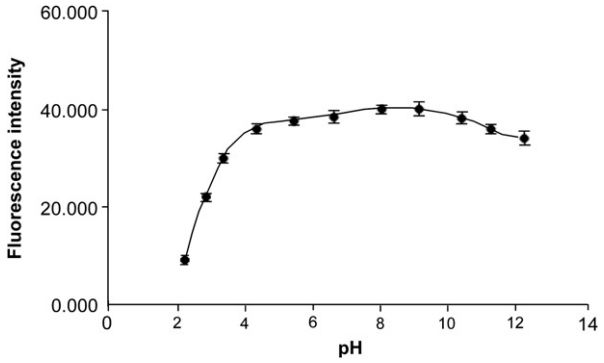

Fig. 6. Effect of pH on the determination of Hg^{2+} with proposed optode (the concentration of Hg^{2+} was fixed at 5.0×10^{-6} mol L^{-1}; error bars were calculated with $n = 5$).

solution. Several compounds including HCl, HNO_3, L-cysteine, ethylenediaminetetraacetic acid (EDTA), thioglycolic acid (TGA), thiourea were tested. The best reagent was 5% thiourea in 1.0 mol L^{-1} HCl solution (acidic thiourea) that gives a short regeneration time (2 min). This is possibly because thiourea behaves as a strong Bronsted acid ($pK_a = -1$ for acidic thiourea) and has a higher complex formation constant ($K_1 = 10^{11.4}$ for Hg^{2+}) than EHT–Hg^{2+} complex.

3.7. Reproducibility and reversibility

The reproducibility and the reversibility of the optode membrane in the determination of mercury was evaluated by repeatedly exposing the optode membrane to a 5.0×10^{-6} mol L^{-1} Hg^{2+} solution and a 5% thiourea in 1.0 mol L^{-1} HCl solution. The sensor was fully reversible and can be regenerated with acidic thiourea solution (Fig. 7). Between the first and eighth cycles, the level of reproducibility of the upper signal level achieved was quite good with a low standard deviation, 68.4 ± 1.9. One sensor film could be used for about 20 repetitive cycles and when kept in a THF-saturated desiccator in dark the same sensor film was found to be stable for at least four months.

The limit of detection (LOD) based on three standard deviations of the blank signal was found to be 1.8×10^{-10} mol L^{-1} (0.036 μg L^{-1}) for Hg^{2+}. This value is lower than that of the mercury ion sensors reported in the literature [11–16,18,19,22–27,35]. The proposed sensor has been determined to have a linear dynamic range of 5.0×10^{-10} and 5.0×10^{-5} mol L^{-1} Hg^{2+}, which may make this technique alternative to the atomic spectrometric techniques.

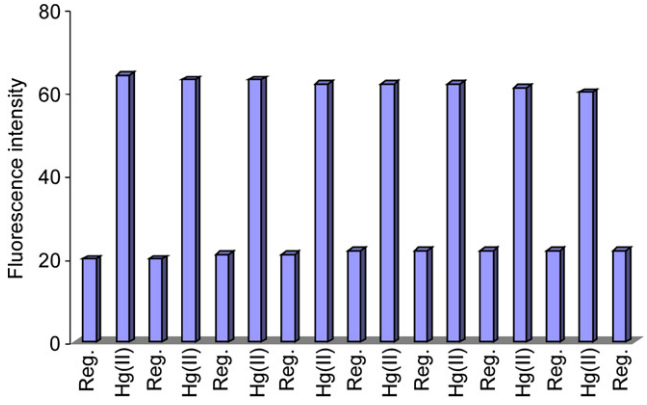

Fig. 7. Variation of the fluorescence of the membrane for repeatedly exposing into 5.0×10^{-6} mol L^{-1} Hg^{2+} solution and 5% thiourea in 1.0 mol L^{-1} HCl solution.

Table 4
Effects of interferent cations on the fluorescence signal of the optical sensor for 5.0×10^{-6} mol L^{-1} Hg^{2+} at pH 5.5.

Interferent[a,b]	Relative error % ($\Delta F/F_0 \times 100$)[c]
Na^+	3.2
K^+	2.9
Ca^{2+}	3.3
Mg^{2+}	3.1
Cu^{2+}	−1.8
Co^{2+}	3.5
Ni^{2+}	2.2
Zn^{2+}	2.9
Cd^{2+}	2.4
Mn^{2+}	4.3
Fe^{3+}	−2.3

[a] Interferent ion concentrations are 1.0×10^{-4} mol L^{-1}.
[b] The concentration of Hg^{2+} is fixed at 5.0×10^{-6} mol L^{-1} (pH 5.5).
[c] ΔF is the difference of fluorescence intensities before and after exposure to interferent cations.

3.8. Selectivity studies

In order to assess the possible analytical application of the sensing method, the effects of various ions on the determination of Hg^{2+} were examined. The data were obtained with a fixed concentration of Hg^{2+} (5.0×10^{-6} mol L^{-1}) and different foreign interferents. The experiments were carried out by recording the change in the fluorescence intensity before (F_0) and after adding the interfering ion at 1.0×10^{-4} mol L^{-1} level (F) into the mercury ion solution buffered at pH 5.5. The resulting relative error (RE) is defined as relative signal change, RE (%) = $[(F - F_0)/F_0] \times 100$. The results of these tests on potential interferences are summarized in Table 4. No significant interferences were observed if a less than ±5% relative error was tolerated.

3.9. Analytical application

In order to assess the usefulness of the proposed method for the determination of Hg^{2+}, it was applied to actual samples of water and hair. Approximately 0.5 g of hair sample was cut with stainless steel scissors from the nape of the neck near the scalp region. Hair washing prior to analysis is required to provide an accurate assessment of endogenous metal content. The washing procedure carried out in this work was the proposed one by the International Atomic Energy Agency [37], using ultrapure water and acetone as washing solvents. The hair samples were decomposed using classic acid digestion method. To carry out the digestion of the samples, 0.3 g of the washed hair samples were accurately weighed into a 100 mL beakers. Then 5 mL of concentrated HNO_3 and 2.5 mL of 30% (v/v) H_2O_2 were added and the mixture was heated on a hot plate for 1 h at 150 °C for complete digestion of the sample. The

Table 5
Determination of mercury(II) concentration in hair and tap water samples ($n = 3$).

Sample	Amount of mercury (μg L^{-1})		Relative error (%)
	Optode	CV-AFS	
Tap water			
1	0.558±0.8	0.533±2.8	4.7
2	0.498±0.6	0.469±2.5	6.2
Hair[a]			
1	220.7±1.2	226.4±4.2	−2.5
2	335.2±1.5	352.1±4.7	−4.8
3	429.5±1.8	415.9±4.9	3.3
4	275.4±1.4	284.5±4.4	3.2

[a] Results are given in μg kg^{-1}.

digests were brought to near dryness, the residue was dissolved in water and made up to 25 mL. Tap water samples were collected from the laboratory.

The results obtained by the proposed method were compared with cold vapour atomic fluorescence spectroscopy (CV-AFS). From the results of three replicate measurements given in Table 5, it is immediately obvious that there is satisfactory agreement between the results obtained by the Hg^{2+} selective optode and by CV-AFS.

4. Conclusions

We have developed a new optical chemical sensor based on a novel triazine-thione derivative in plasticized PVC membrane for the determination of Hg^{2+} ions, with good optical and mechanical properties. The optode is fully reversible, highly selective and can be easily regenerated with acidic thiourea solution. A very low LOD, 1.8×10^{-10} mol L^{-1}, was reached. The proposed optode has a wide dynamic range, a reproducible response and provides an inexpensive and quick method for the determination of Hg^{2+}. The sensor was applied successfully to the determination of mercury in real samples.

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