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A Simple and Highly Structured Procaine Hydrochloride as Fluorescent Quenching Chemosensor for Trace Determination of Mercury Species in Water

Dyab A. Al-Eryani, Waqas Ahmad, Zeinab M. Saigl,
Hassan Alwael, Saleh O. Bahaffi,
Yousry M. Moustafa and Mohammad S. El-Shahawi

Additional information is available at the end of the chapter

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Abstract

An ultrasensitive, simple and highly selective spectrofluorometric strategy for quantifying traces of mercury(II) in environmental water has been established using the fluorescent probe procaine hydrochloride ($PQ^+.Cl^-$). The procedure was based upon the formation of the ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ between $PQ^+.Cl^-$ and mercury(II) in iodide media at pH 9.0–10.0 with its subsequent extraction onto dichloromethane accompanied by a change in fluorescence intensity at $\lambda_{ex/em} = 268/333$ nm. The developed strategy exhibited a linear range of 1–114 $\mu g L^{-1}$ with lower limit of detection (LOD) and quantification (LOQ) of mercury(II) 1.3 and 3.98 nM, respectively. Intra and inter-day laboratory accuracy and precision for trace analysis of mercury(II) in water were performed. Complexed mercury(II) in environmental water, chemical speciation and successful literature comparison was performed. The proposed system offered excellent selectivity towards mercury(II) ions examined in the presence of competent ions in excess, relevant to real water samples. The method was applied for analysis of mercury(II) in tap water samples. Statistical comparison (Student's *t* and *F* tests) of the proposed method with the reference ICP-OES method revealed no significant differences in the accuracy and precision.

Keywords: spectrofluorometry, mercury(II), fluorescence, procaine hydrochloride, quenching, ternary ion associate complex

1. Introduction

Heavy metal pollution is global level environmental concern, which poses serious implications towards human health [1, 2]. Heavy metal pollution has attained considerable interest

in the recent past [3]. Among heavy metals, mercury is considered among the most toxic and dangerous ion due to its wide existence as an ore cinnabar in nature and its applications as pigment vermilion, detoxification/anticorrosive medicines and mercury fulminate detonator in explosives [3, 4]. Due to its wide presence in environment, it enters the biological membranes through respiratory and gastrointestinal tissues [4, 5]. It can also cause permanent harm to the endocrine and central nervous systems if accumulated in the food chain and ultimately in human body [5–13]. Moreover, a low-level exposure of mercury will affect the endocrine and nervous systems, brain and kidneys [14, 15].

Several forms of mercury including elemental, organic and inorganic or elemental display different levels of toxicity and contamination in natural water resources and drinking water [2]. The most stable and prevalent form of mercury that contributes to wider contamination is the solvated divalent mercuric ion (Hg^{2+}), due to its high solubility in water [16]. The US Environmental Protection Agency (US-EPA) and World Health Organization (WHO) have set the permissible level (MPL) for mercury to 10 nM [1, 17]. Traces of mercury ions have shown significant toxicity, and therefore developing highly sensitive methodologies are considered essential [18]. Thus, recent trends have been oriented towards developing highly sensitive and selective procedures for monitoring and/or enrichment of mercury in various water samples [20–22].

Several analytical procedures, including atomic absorption and emission spectrometry (AAS, AES) [19, 20], inductively coupled plasma-optical emission spectrometry (ICP-OES) [21], ion exchange chromatography [22], mechanical filtration [23], chemical precipitation [24], reverse osmosis, flotation [25, 26] and membrane separation [27], are reported for mercury determination. On the other hand, numerous liquid and solid phase extraction methods such as liquid-liquid extraction (LLE) [28], carbon nanotubes [29], graphene oxide [30–33] and polymers [34–36] have been reported for routine analysis of mercury(II). However, most of these methods require sophisticated equipment unavailable for use in developing countries with numerous other limitations like high cost, complications in their proper operation, selectivity and sensitivity [34, 35].

Recently, several molecular probe-based sensors using organic chromophores, quantum dots (QDs), small fluorescent organic molecules, proteins, antibodies and conjugated polymers coupled with several spectrometric and electrochemical techniques are reported for mercury(II) determination [36–53]. Some of these methods suffer from solubility issues, low stability, lower sensitivity and selectivity, complicated synthesis procedures and environmentally unfriendliness to monitor mercury(II) in biological and environmental samples. Hence, the work presented in this chapter is focused on: (i) developing a simple, highly selective and sensitive extractive spectrofluorometric LLE for trace determination of mercury(II) species in water samples using the ion pairing reagent 4-amino-N-(2-diethylaminoethyl) benzamide hydrochloride namely procaine hydrochloride and abbreviated as (PQ^+Cl^-) (**Figure 1**) [54–59]; (ii) the utility of the proposed extractive LLE for trace determination of mercury ions in environmental water samples and finally (iii) validation and assignment of the most probable stoichiometry and chemical equilibria and fluorescence quenching mechanism of the produced ternary ion associate complex $[(\text{PQ}^+)_2 \cdot (\text{HgI}_4)^{2-}]$ of $[\text{HgI}_4]^{2-}$ with the proposed ion pairing reagent [60–64].

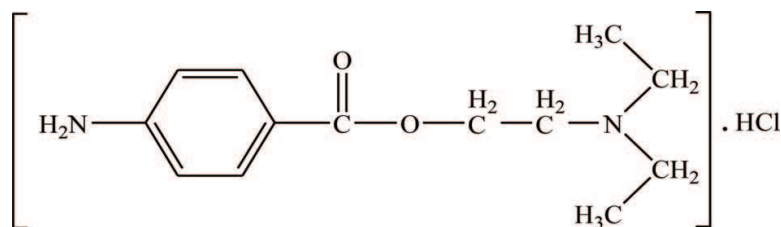


Figure 1. Chemical structure of procaine hydrochloride.

2. Experimental

2.1. Reagents and materials

Glasswares were pre-cleaned with HNO_3 (20% m/v), acetone and deionized water. Analytical reagent grade chemicals and solvents were used as received. Low-density polyethylene (LDPE) bottles, Nalgene were used for storage of the stock solutions. A stock solution (1.0 mg mL^{-1}) of standard mercury(II) solution was prepared in ultra-pure water. Stock solution ($1000 \mu\text{M}$) of mercury(II) was prepared from HgCl_2 (BDH Chemicals, Poole, England) in ultra-pure water. More dilute solutions of mercury(II) ions were prepared by series dilution in deionized water. Potassium iodide solution (10% w/v) was prepared in ultra-pure water. Stock solutions of other cations were synthesized either from their chloride or nitrate salts in deionized water. Britton-Robinson (B-R) (pH 2–11) buffers were prepared from BDH purchased H_3BO_3 , H_3PO_4 , CH_3COOH and NaOH in deionized water as reported [65]. A stock solution ($1000 \mu\text{M}$) of procaine hydrochloride (Sigma–Aldrich) was prepared by dissolving the required weight in aqueous solution.

2.2. Apparatus

Fluorescent measurements were recorded on a Perkin-Elmer LS55 spectrofluorometer, USA equipped with quartz cuvettes of path length $10 \times 10 \text{ mm}$. UV-visible (190–1100 nm) spectra were recorded on a Perkin-Elmer spectrophotometer (Lambda 25, Shelton, CT, USA). For method validation, a Perkin-Elmer inductively coupled plasma-optical emission spectrometry (ICP-OES) (California, CT, USA) was utilized for mercury determination at the optimum operational parameters (**Table 1**). Deionized water was obtained from Milli-Q Plus system (Millipore, Bedford, MA, USA). A digital micropipette (Plus-Sed) and pH meter (inoLap pH/ion level 2) were used for preparation of stock and more diluted solutions of reagent and mercury(II) and pH measurements, respectively.

2.3. Recommended procedures

2.3.1. Spectrophotometric procedure

Appropriate volume (1.0 mL) of various mercury(II) concentrations (1.0×10^{-5} to $8 \times 10^{-5} \text{ M}$) was transferred to a series of glass test tubes (10.0 mL) followed by addition of $100 \mu\text{L}$ KI (10% w/v). The solutions were made up to the mark with Britton-Robinson (B-R) buffer solution of pH 10 after

Parameter	Unit
RF power	1400 W
Nebulizer flow	0.7 L/min
Auxiliary flow	0.3 L/min
Plasma flow	10.0 L/min
Sample pump flow	1 mL/min
Plasma viewing	Axial
Processing mode	Area
Replicates	3
Nebulizer type	Cross-flow (Gim Tip)
Spray chamber	Scott (Ryton)
Injector	Scott (Ryton)
Analytical wavelength	Hg 194.2 nm

Table 1. Operational parameters of ICP-OES for analysis of mercury.

adding 1.0 mL (1.0×10^{-4} M) of procaine hydrochloride. The solution mixtures were shaken well for 3.0 min with dichloromethane (5.0 mL). After equilibrium, the organic phase was separated out, shaken with anhydrous Na_2SO_4 to remove trace of water and finally the absorbance of the organic extract was finally measured at λ_{max} 310 nm *versus* the reagent blank at room temperature.

2.3.2. Spectrofluorometric procedure

In a series of glass test tubes (10.0 mL), appropriate concentrations (20–140 nM) of mercury(II) and 100 μL of 10% KI (w/v) were added. The test solutions were completed to the mark with B-R buffer of pH 10.0 after adding 1.0 mL (20 μM) of $\text{PQ}^+\cdot\text{Cl}^-$ and shaken well for 3.0 min with dichloromethane (5.0 mL). The organic phase was separated after equilibrium, shaken with anhydrous Na_2SO_4 and analyzed at $\lambda_{\text{ex/em}}$ = 268/333 nm *versus* a reagent blank at room temperature. The quenched fluorescence in signal intensity (ΔF) of $\text{PQ}^+\cdot\text{Cl}^-$ by mercury(II) was computed employing the following equation:

$$\Delta F = F_0 - F \quad (1)$$

where F_0 and F are the fluorescence intensities of the reagent before and after addition of mercury(II) under the optimized analytical parameters, respectively. The fluorescence signal intensity (F) of the formed ion associate complex was measured *versus* a reagent blank as described above. The quenched fluorescence (ΔF) was finally determined. Following the recommended procedures, the selectivity of the proposed method was examined in the presence of a series of the concurrent diverse ions, e.g. Ca^{2+} , Ba^{2+} , Cu^{2+} , Pb^{2+} , Fe^{3+} , As^{3+} , Ag^+ , Al^{3+} , Sn^{2+} , Cd^{2+} , Bi^{3+} , WO_4^{2-} , MnO_4^- , F^- , CO_3^{2-} , SO_4^{2-} in the presence of mercury(II) at $2.0 \mu\text{g L}^{-1}$.

2.3.3. Calibration curve of mercury(II)

To construct the linear calibration plot, a series of solutions (10.0 mL) containing various known (5.0 mL, 20–140 nM) concentrations of mercury(II) and $PQ^+.Cl^-$ (1.0 mL, 20 μ M) were transferred to measuring flask (25 mL) prepared. The test solutions were completed to the mark with B-R buffer of pH 10 and shaken well with dichloromethane (5.0 mL) for 3.0 min. After equilibrium, the organic phase was separated out, shaken with anhydrous Na_2SO_4 and analyzed at $\lambda_{ex/em} = 268/333$ nm *versus* a reagent blank at room temperature. The quenched fluorescence signal intensity (ΔF) of the formed colorless complex species was finally measured *versus* a reagent blank as described above.

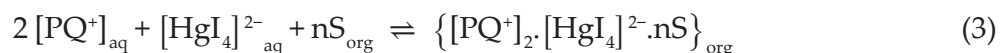
2.4. Analytical applications

In LDPE sample bottles, pre-cleaned as described in Section 3.2.1, tap water samples were collected from the laboratories of the Department of Chemistry, King Abdul Aziz University, Jeddah, KSA and immediately filtered through 0.25 μ m cellulose membrane filters before analysis and stored in LDPE bottles. The sample solutions were then spiked with known concentration (20–100 nM) of mercury(II). The fluorescence intensity of the test solutions were measured at ($\lambda_{ex/em} = 268/333$ nm) under the optimized experimental conditions of mercury(II) by standard addition plot. The concentration and the percent recovery of the mercury(II) added to water samples were finally computed.

3. Results and discussion

3.1. Electronic and fluorescence spectra of reagent and its mercury(II) ion associate complex

The absorption spectrum of the ion pairing reagent $PQ^+.Cl^-$ is demonstrated in **Figure 2**. The spectrum showed one well-defined band at 290 nm and was safely assigned to $n \rightarrow \pi^*$ electronic transitions [66, 67]. The electronic absorption spectra of the developed ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ of $PQ^+.Cl^-$ (4×10^{-5} M) with mercury(II) (1×10^{-5} M) in the presence of an excess aqueous KI (10% w/v) were recorded and a well-defined absorption peak at 310 nm was noticed (**Figure 2**). The most probable formation mechanism of ion associate complex of mercury can be expressed as follows [68–73]:



As shown in **Figure 3**, the solution of reagent blank $PQ^+.Cl^-$ with concentration of 5.0 μ M exhibits a strong fluorescence intensity at $\lambda_{ex/em} = 268/333$ nm in dichloromethane [68, 74–76]. The strong fluorescence band of $PQ^+.Cl^-$ can be quenched after addition of 40 nM Hg(II) and increases after adding excess of mercury(II), through the formation of ternary ion associate

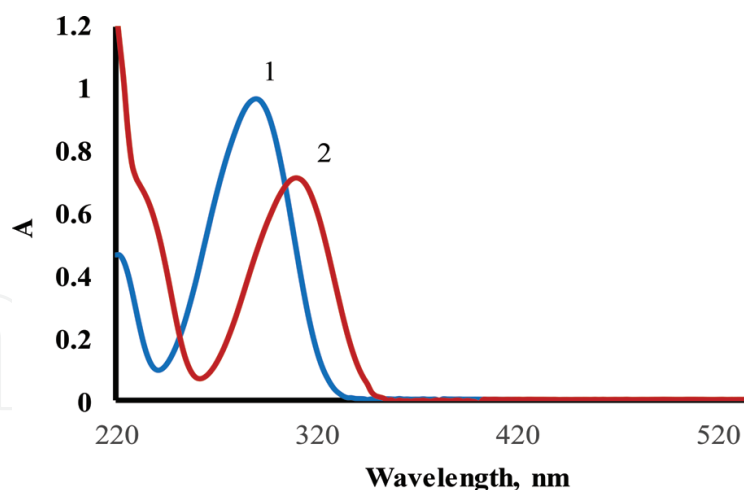


Figure 2. Electronic spectra of the $\text{PQ}^+.\text{Cl}^-$ (4×10^{-5} M) (1) and its ternary ion associate complex $[(\text{PQ}^+)_2.(\text{HgI}_4)^{2-}]$ in dichloromethane (2). Condition: Mercury (1×10^{-5} M).

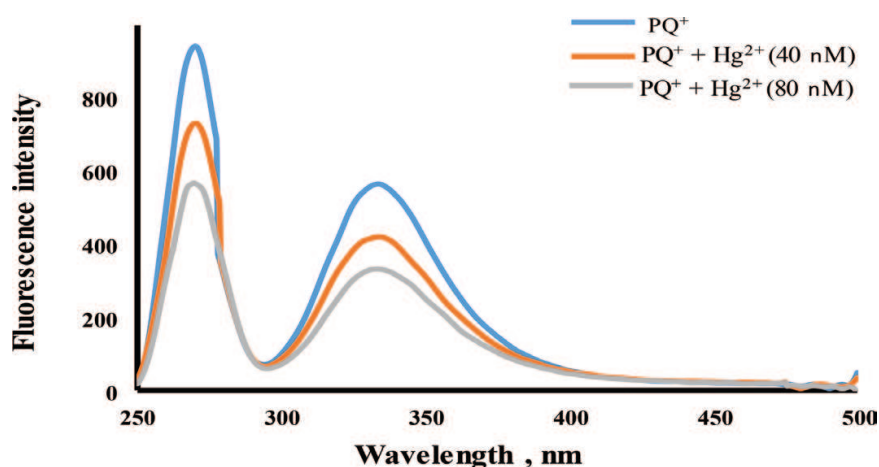


Figure 3. Fluorescence spectra of $\text{PQ}^+.\text{Cl}^-$ ($5.0 \mu\text{M}$) and its ternary ion associate complex $[(\text{PQ}^+)_2.(\text{HgI}_4)^{2-}]$ with mercury(II) ions (40, 80 nM).

complex $[(\text{PQ}^+)_2.(\text{HgI}_4)^{2-}]$ (Figure 3) between $\text{PQ}^+.\text{Cl}^-$ and HgI_4^{2-} [60]. Hence, this sensing principle by virtue of quenching was successfully applied for sensitive spectrofluorometric determination of mercury(II).

3.2. Analytical parameters

The effect of various experimental conditions on the fluorescence characteristics of both ion associate complex was studied, including type of solvents, pH, reagent concentration and equilibrium time.

3.2.1. Effect of solvent

The influence of extraction solvent, e.g. n-hexane, chloroform, dichloromethane, toluene and cyclohexane, on the fluorescence quenching of the formed mercury(II) complex was studied. The results are demonstrated in Figure 4. Stable and maximum change in fluorescence

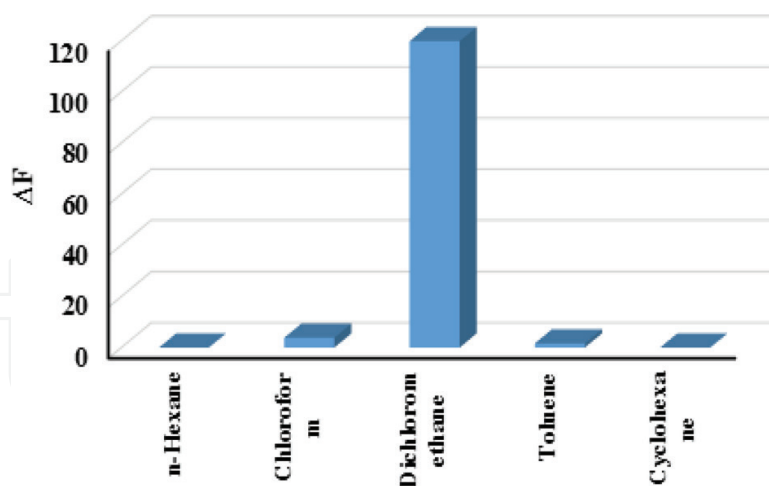


Figure 4. Influence of type extraction solvent on the fluorescence quenching of $PQ^+.Cl^-$ ($5.0 \mu M$) reagent by $K_2[HgI_4]$ ($80 nM$).

quenching was observed in dichloromethane. Therefore, a detailed study on the influence of dichloromethane volume on the fluorescence quenching of $PQ^+.Cl^-$ reagent by mercury(II) was critically studied (**Figure 5**). Thus, dichloromethane was adopted at $2.0 mL$ volume in the subsequent work pertaining to its higher performance compared to other volume fractions.

3.2.2. Effect of pH

The effect of pH on the fluorescence quenching of the formed mercury(II)- PQ^+ complex was studied in B-R (pH 3–12) buffer solutions. Maximum quenching by mercury(II) quencher was achieved at $pH \approx 9-10$. The extraction rate at $pH < 10.0$ for ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ was lower. Several factors including hydrolysis, instability and/or incomplete extraction, and the slow dissociation of the complex $K_2[HgI_4]^{2-}$ account for the decrease in the amount of mercury extracted at pH values other than $pH \approx 9-10$ [62, 63]. Thus, in the next work, the solution media were adopted at $pH \approx 10$ due to the ease in formation of the ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$.

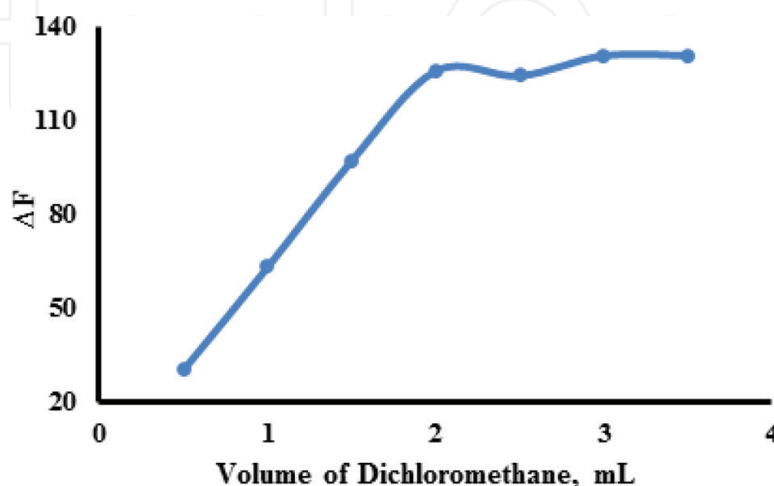


Figure 5. Influence of dichloromethane volume on the fluorescence quenching of $PQ^+.Cl^-$ ($5.0 \mu M$) reagent by mercury ($80 nM$).

3.2.3. Effect of extraction time

The stability and the fluorescence signal intensity of the emission spectrum of the formed ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ considerably depend on the reaction time. Therefore, the fluorescence intensity of the emission spectrum of the ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ was measured at various time intervals (0.5–14 min) at $\lambda_{ex/em} = 268/333$ nm at the optimum conditions. The results are demonstrated in **Figure 6**. Maximum stability in signal intensity was achieved after 2.0 min and remained constant for longer time up to 14.0 min. Therefore, a standing time of 2.0 min was adopted in the subsequent work.

3.2.4. Effect of $PQ^+.Cl^-$ concentration

The influence of $PQ^+.Cl^-$ concentration on the fluorescence quenching of mercury(II) at concentration of 80 nM was studied in KI (0.1% w/v). Thus, various fractions of $PQ^+.Cl^-$ solution were added to $[HgI_4]^{2-}$. The fluorescence quenching (ΔF) increased on increasing $PQ^+.Cl^-$ concentration up to 5.0 μM and leveled off at higher concentration. This possible self-absorption and aggregation of the reagent at high concentration contribute to this enhanced behavior [77]. Thus, in the subsequent work, a concentration of 5.0 μM of $PQ^+.Cl^-$ was selected, capable of successfully quantifying the target up to trace level proportions.

3.3. Selectivity

Microenvironment resembling real sample matrix containing competent interfering ion was designed to critically examine the applicability of the proposed method. Thus, the quenching in the fluorescence intensity of the ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ before and after adding the interfering ions in the presence of relatively high concentration (50–500 $\mu g L^{-1}$) of other metal ions (K^+ , Ca^{2+} , Ba^{2+} , Cu^{2+} , Pb^{2+} , Fe^{3+} , As^{3+} , Ag^+ , Al^{3+} , Sn^{2+} , Cd^{2+} , Bi^{3+} , WO_4^{2-} , MnO_4^- , F^- , CO_3^{2-} , SO_4^{2-}) under the same condition was studied. The results are shown in **Figure 7**, where no noticeable quenching changes were observed. The data indicated that

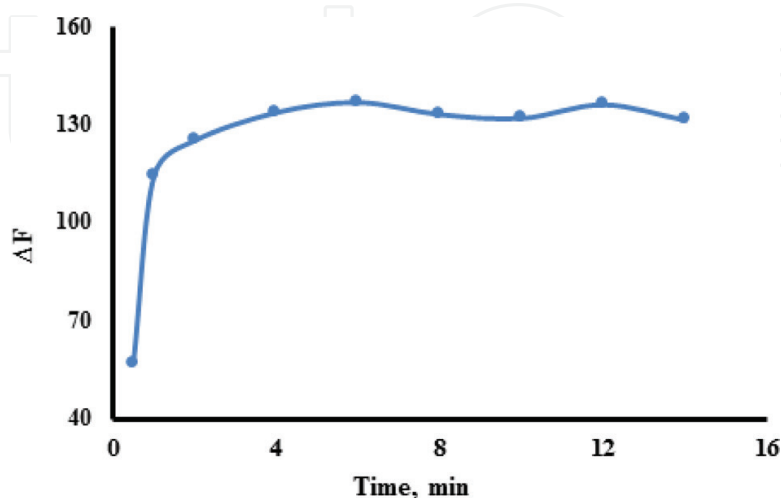


Figure 6. Plot of time vs. the fluorescence quenching of $PQ^+.Cl^-$ (5.0 μM) reagent by mercury (80 nM).

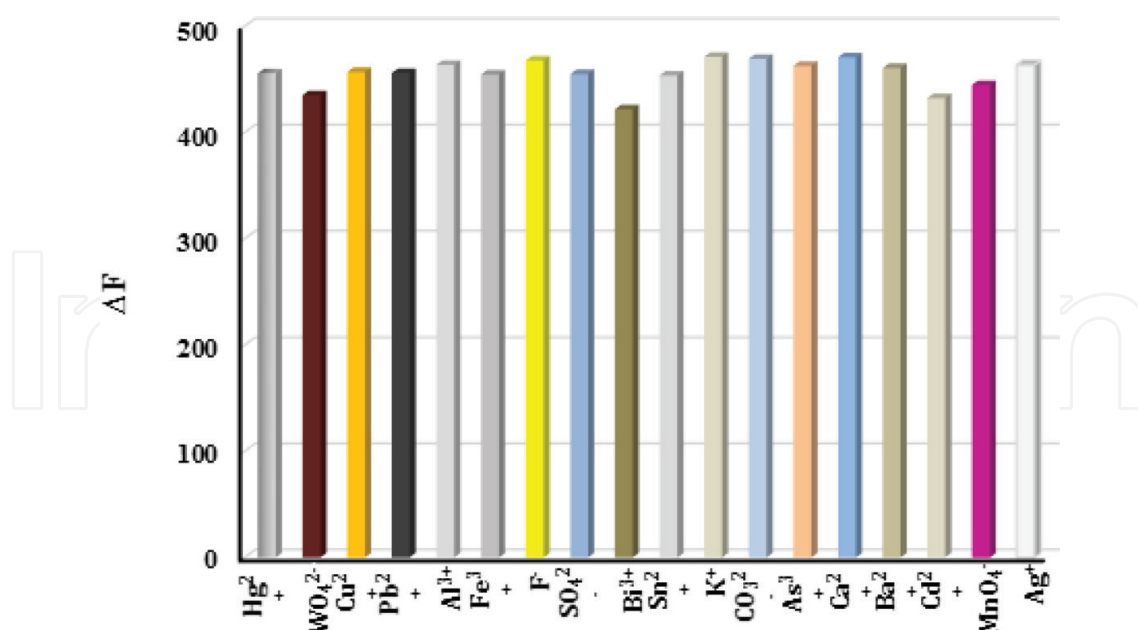


Figure 7. The values of quenching fluorescence intensity of PQ⁺.Cl⁻ (5.0 μg/L) with addition of Hg(II) (2.0 μg/L) in the presence of different metal ions (500 μg/L) in KI (0.1% w/v).

PQ⁺.Cl⁻ possess good selectivity in probing mercury(II). As shown in **Figure 7**, the signal intensity in the presence of 2.0 μg L⁻¹ mercury(II) mixed with 500 μg L⁻¹ of other metal ions in KI (0.1% w/v) was found similar to mercury(II) alone at 2.0 μg L⁻¹ in KI (0.1% w/v). Thus, it can be concluded that the probe is selective towards mercury(II) in the presence of potent interfering ions in higher concentration.

3.4. Analytical performance

Under the optimized experimental conditions, the influence of various known mercury(II) concentrations (20–140 nM) on the fluorescence spectra of mercury(II)-PQ⁺ was recorded. The plot of mercury(II) concentrations (20–140 nM) *versus* ΔF was linear (**Figure 8**) with the following regression equation:

$$\Delta F = 1.6354 [C] \text{ (nM)} - 3.3971 \quad (4)$$

where C and ΔF are the concentration of mercury(II) ions and quenched fluorescence, respectively.

The precision of the method was checked by means of ANOVA by performing five successive replicates per day for 5 days for a typical sample containing 100 nM mercury(II) (**Table 2**).

The precision of the proposed method was evaluated. The calculated value of F (F = 1.64) (**Table 3**) was found lower than the tabulated F value (F = 2.87) at 95% confidence level [78]. The obtained LOD and LOQ [78] for the proposed method for mercury(II) species were 1.3 and 3.98 nM, respectively. This value was significantly lower than the maximum allowable mercury concentration (10.0 nM) by USEPA in drinking water [17]. The proposed method

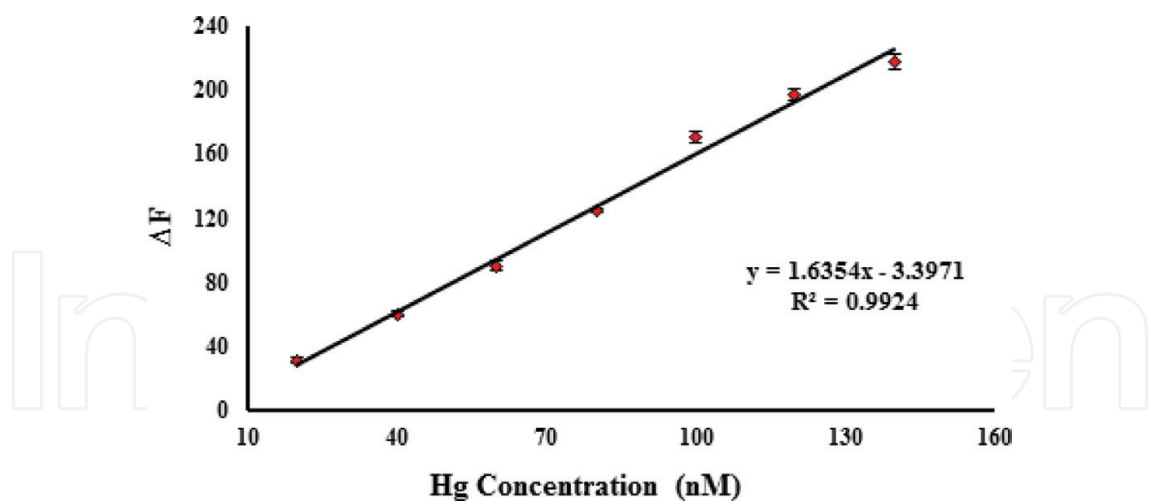


Figure 8. Calibration plot of ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$. Conditions: Mercury(II) (20–140 nM), PQ^+ (5.0 μ M) and KI (0.1% w/v) pH \approx 10 at $\lambda_{ex/em}$ = 268/333 nm.

could be considered for routine analysis of mercury due to high precision and selectivity in real samples in the presence of excess relevant competent ions. Moreover, the utility of the proposed method was finally evaluated by comparing the analytical features of the proposed method with a wide range of promising studies in literature. It includes successful comparison with a series of published fluorescence [30, 37–41, 43], electrochemical sensor [42], spectrophotometric [44, 48], colorimetry [45, 49], chemiluminescence [46], single-crystal X-ray diffraction [47], electrochemiluminescence biosensor [50], X-ray fluorescence (XRF) [51], differential pulse voltammetry [36, 52], ICP-OES [21], electrochemical [50] and X-ray photoelectron spectroscopy (XPS) [53], in terms of LOD and LOQ (**Table 4**).

3.5. Fluorescence quenching mechanism

The fluorescence quenching process of $PQ^+.Cl^-$ by mercury(II) as a quencher was critically investigated to evaluate the fluorescence mechanism for the formed ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ [60, 62–64]. The fluorescence spectra of $PQ^+.Cl^-$ ($\lambda_{ex}/\lambda_{em}$ 268/330 nm)

Replicate	First day ΔF	Second day ΔF	Third day ΔF	Fourth day ΔF	Fifth day ΔF
1	167.00	163.88	166.10	159.40	167.59
2	164.47	168.70	163.75	162.32	165.55
3	161.81	166.43	167.99	166.78	166.34
4	160.53	166.45	161.69	164.76	164.00
5	165.70	162.44	165.08	161.13	169.45
Mean	163.90	165.58	164.92	162.88	166.59
SD	2.69	2.45	2.38	2.93	2.06

Table 2. Five days and five replicates per day determined the quenching fluorescence intensity of the reagent $PQ.Cl^-$ by mercury(II) (100 nM) added.

Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F value S_1/S_2
Between days	41.55	4	10.39	1.64
Within days	126.77	20	6.34	
Total	168.32	24		

[†] S_1 : Regression mean square. S_2 : Mean square error.

Table 3. Analysis of variance (ANOVA) for the linear equation results[†].

Technique	Reagent	LOD	Ref
Fluorescence	(2-Pyridylmethyl)(2-quinolylmethyl) amine	2.6×10^{-8} M	[37]
Fluorescence	Rhodamine hydrazine and 2-hydroxy-acetophenone	150×10^{-9} M	[38]
Fluorescence	Phenylamine-oligothiophene	4.39×10^{-7} M	[39]
Fluorescence	Squaraine-bis(rhodamine-B)	6.47×10^{-6} M	[40]
Fluorescence	Rhodamine (R-2)	1×10^{-8} M	[41]
Electrochemical sensor	DNA-generated gold amalgam	0.002×10^{-9} M	[42]
Fluorescence	Rhodamine 6G derivative and AuNPs	0.75×10^{-9} M	[43]
Fluorescence	1,4-Bis(styryl)benzene	7×10^{-9} M	[44]
Colorimetry	Au-NPs	0.0084×10^{-9} M	[45]
Chemiluminescence	Rhodamine B and gold nanoprisms	0.027×10^{-9} M	[46]
Single-crystal X-ray diffraction	1-[(5-Benzyl-1,3-thiazol-2-yl) diazenyl] naphthalene-2-ol	0.41×10^{-6} M	[47]
Spectrophotometric	bis(2-Ethylhexyl) phosphate	3.5×10^{-9} M	[48]
Colorimetry	Carrageenan-functionalized Ag/AgCl nanoparticles	1×10^{-6} M	[49]
Electrochemiluminescence biosensor	tris-(Bipyridine) (Ru(bpy) ₃ ²⁺)/cyclodextrins-Au nanoparticles(CD-AuNps)/Nafion	0.1×10^{-9} M	[50]
X-ray fluorescence (XRF)	—	37×10^{-6} M	[51]
Differential pulse voltammetry	Polypyrrole decorated graphene/ β -cyclodextrin	0.47×10^{-9} M	[36]
Inductively coupled plasma-optical emission spectrometry (ICP-OES)	—	0.15×10^{-9} M	[21]
Differential pulse voltammetry	Copper cobalt hexacyanoferrate	80×10^{-9} M	[52]
Electrochemical and X-ray photoelectron spectroscopy(XPS)	1-Undecanethiol assembled Au substrate	4.5×10^{-9} M	[53]
Fluorescence	DNA-functionalized-graphene	4.1×10^{-9} M	[30]
Spectrofluorometry	Procaine hydrochloride	1.3×10^{-9} M	Present work

Table 4. Analytical features of different methods employed for mercury(II) determination.

upon introduction of varying concentrations ($0.3\text{--}1.0\ \mu\text{g L}^{-1}$) of quencher (mercury(II) ions) are shown in **Figure 9**. The fluorescence intensity of $\text{PQ}^+\cdot\text{Cl}^-$ decreases regularly with increasing quencher concentration. The Stern-Volmer (K_{SV}) constant was calculated by employing the equation:

$$F_0/F = 1 + K_{\text{SV}} [Q] \quad (5)$$

where F_0 and F are the fluorescence signals in the absence and presence of $[\text{HgI}_4]^{2-}$ quencher, respectively. K_{SV} is the Stern-Volmer constant and $[Q]$ is the quencher concentration. The values of K_{SV} and correlation factor calculated by plotting fluorescence quenching (ΔF) of $\text{PQ}^+\cdot\text{Cl}^-$ versus $[\text{Hg}^{2+}]$ were found equal to $1.87 \times 10^6\ \text{L g}^{-1}\ \text{mol}^{-1}$ and 0.9909, respectively.

The chemical composition of the ternary ion associate complex $[(\text{PQ}^+)_2(\text{HgI}_4)^{2-}]$ was determined from the Benesi-Hildebrand linear model by employing the following equation [77, 79–82]:

$$\frac{1}{(F_0 - F)} = \frac{1}{(F - F_\infty)} + \frac{1}{(F - F_\infty) \times K \times [Q]^2} \quad (6)$$

where F_∞ represents the emission intensity of the ternary ion associate complex $[(\text{PQ}^+)_2(\text{HgI}_4)^{2-}]$ at equilibrium and K is association constant. The number of the binding sites (n) and the apparent binding constant (K) of $\text{PQ}^+\cdot\text{Cl}^-$ that independently binds to equivalent sites on a macromolecule were determined from the linear plot of Benesi-Hildebrand ($1/(I - I_0)$ versus $1/([\text{Mercury(II)}])$). The plot revealed formation of 1:2 stoichiometry of $[\text{HgI}_4]^{2-}:\text{PQ}^+\cdot\text{Cl}^-$ molar ratio in the produced ternary ion associate complex $[(\text{PQ}^+)_2(\text{HgI}_4)^{2-}]$. The calculated association constant K was found equal to $73\ \text{M}^{-1}$.

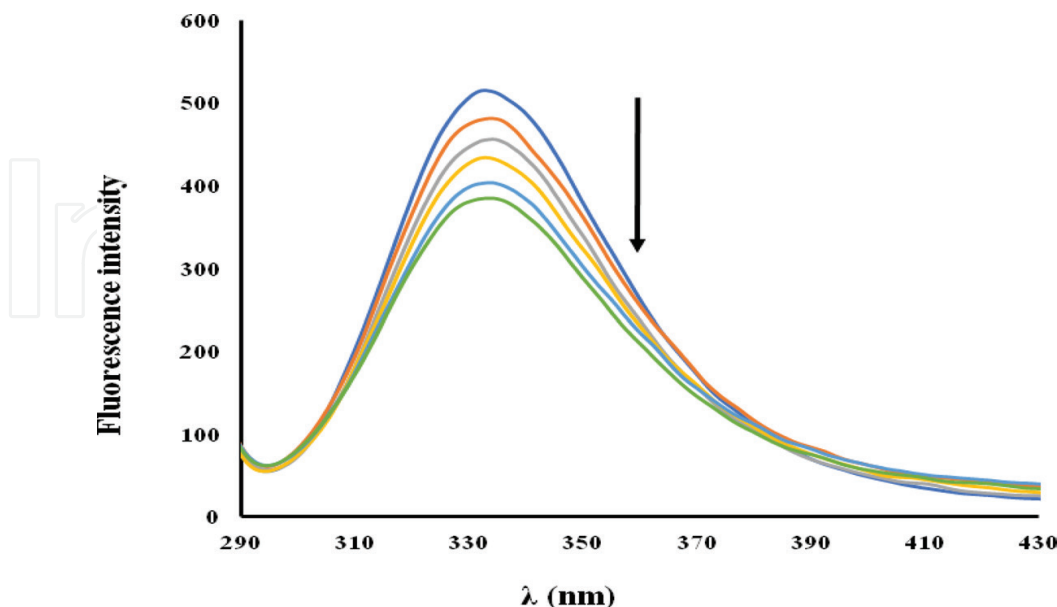


Figure 9. Fluorescence quenching spectra of $\text{PQ}^+\cdot\text{Cl}^-$ ($5.0\ \mu\text{M}$) in the presence of various concentrations ($20\text{--}80\ \text{nM}$) of the mercury(II) ions. Conditions: KI (0.1% w/v) $\text{pH} \approx 10$, at $\lambda_{\text{ex/em}} = 268/333\ \text{nm}$.

Samples	Spiked (nM/L)	Proposed method	Proposed PQ+ method	ICP-OES	
		Detected (nM) mean \pm SD	Recovery (%)	Detected (nM) mean \pm SD	Recovery (%)
Tap water 1	20	20.97 \pm 0.50	104.7	20.54 \pm 0.63	102.7
Tap water 2	40	39.11 \pm 1.25	97.78	39.22 \pm 0.92	98.05
Tap water 3	60	61.74 \pm 3.07	102.23	62.58 \pm 0.72	104.3
Tap water 4	80	78.51 \pm 1.63	98.14	77.88 \pm 1.21	97.35
Tap water 5	100	97.74 \pm 3.51	97.74	98.37 \pm 1.30	98.37

[†]Average \pm standard deviation (n = 5).

Table 5. Analytical data mercury(II) assay by the developed and ICP-OES methods[†].

3.6. Analytical applications

Applications of the proposed method were tested for analysis of mercury(II) in tap water samples (King Abdulaziz University, Jeddah, KSA). Samples were spiked with known concentrations (20–100 nM) of mercury(II) ion and analyzed by the developed method. In each sample, the fluorescence quenching of PQ⁺.Cl[−] was immediately measured after spiking of mercury(II) onto the water samples. For method validation, mercury(II) concentrations in the spiked samples were also determined by the standard ICP-OES method. The results are summarized in **Table 5**. The recovery percentage of the measured mercury(II) added to the real samples by the developed and the standard ICP-OES methods was in the range from 97.74 to 104.7% and 98.05 to 102.7%, respectively. The calculated values of the Student's *t* and *F* tests were found lower than the tabulated Student's *t* and *F* tests at 0.05 probability [78] revealing no significant differences between both methods. Thus, it can be concluded that the proposed fluorescence probe can be used as a potential assay for sensing of mercury(II) in complex matrices.

4. Conclusion

A new and facile extractive spectrofluorometric method for cost effective, precise, accurate and selective determination of trace levels of mercury(II) in water. The proposed method was based upon formation of ternary ion associate complex [(PQ⁺)₂.(HgI₄)^{2−}] between [HgI₄]^{2−} and the fluorescence probe PQ⁺.Cl[−]. The proposed system offered excellent selectivity towards mercury(II) ions over most anions and metal cations. The detection process could be performed quickly at room temperature without any catalyst or oxidizer. The proposed method provides LOD (1.3 nM) lower than the value set by WHO (10 nM) and USEPA for drinkable water [1, 17]. The developed method is easy to operate as it does not require sophisticated experimental techniques, and the proposed assay is useful for point-of care applications. Moreover, the method opens capable ways for developing fluorescence assay strategies. The proposed approach was validated successfully by analysis of mercury(II) in environmental

water samples by ICP-OES data and statistical treatment of data in terms of significant tests, e.g. *F* and Student's *t* tests. The method could be expanded for ultra-trace analysis of mercury(II) ions in water after on-line enrichment on nanosized solid phase extractor, e.g. polyurethane foam packed column followed by elution with selective reagent [83] and/or its proposed coupling with the advanced microextraction techniques [84]. Therefore, the present work suggested the suitability of the proposed method for use in routine analysis and applicable strategy for analysis of mercury(II) in complex matrices.

Author details

Dyab A. Al-Eryani^{1,2}, Waqas Ahmad¹, Zeinab M. Saigl¹, Hassan Alwael¹, Saleh O. Bahaffi¹, Yousry M. Moustafa³ and Mohammad S. El-Shahawi^{1,4†*}

*Address all correspondence to: mohammad_el_shahawi@yahoo.co.uk

1 Department of Chemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

2 Department of Chemistry, Faculty of Applied Science, Tamar University, Tamar, Yemen

3 Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia

4 Department of Chemistry, Faculty of Science, Damiatta University, Damiatta, Egypt

†The author is on sabbatical leave from the Department of Chemistry, Faculty of Science, Damiatta University, Damiatta, Egypt.

References

- [1] World Health Organization (WHO). Guidelines for Drinking Water, 4th ed. Switzerland; 2011
- [2] Elinder CG, Gerhardsson L, Oberdoerster G. Biological monitoring of toxic metals-overview. In: Biological Monitoring of Toxic Metals. US: Springer; 1988. pp. 1-71
- [3] Aragay G, Pons J, Merkoci A. Recent trends in macro-, micro-, and nanomaterial-based tools and strategies for heavy-metal detection. Chemical Reviews. 2011;**111**:3433-3458
- [4] Lin YH, Tseng WL. Ultrasensitive sensing of Hg²⁺ and CH₃Hg⁺ based on the fluorescence quenching of lysozyme type VI-stabilized gold nanoclusters. Analytical Chemistry. 2010;**82**:9194-9200
- [5] Wen D, Deng L, Guo SJ, Dong SJ. Self-powered sensor for trace Hg²⁺ detection. Analytical Chemistry. 2011;**83**:3968-3972
- [6] Ye BC, Yin BC. Highly sensitive detection of mercury(II) ions by fluorescence polarization enhanced by gold nanoparticles. Angewandte Chemie, International Edition. 2008;**47**:8386-8389

- [7] Liu DB, Qu WS, Chen WW, Zhang W, Wang Z, Jiang XY. Highly sensitive, colorimetric detection of mercury(II) in aqueous media by quaternary ammonium group-capped gold nanoparticles at room temperature. *Analytical Chemistry*. 2010;**82**:9606-9610
- [8] Deng L, Ouyang XY, Jin JY, Ma C, Jiang Y, Zheng J, Li JS, Li YH, Tan WH, Yang RH. Exploiting the higher specificity of silver amalgamation: Selective detection of mercury(II) by forming Ag/hg amalgam. *Analytical Chemistry*. 2013;**85**:8594-8600
- [9] Wei H, Wang ZD, Yang LM, Tian SL, Hou CJ, Lu Y. Lysozyme-stabilized gold fluorescent cluster: Synthesis and application as Hg²⁺ sensor. *Analyst*. 2010;**135**:1406-1410
- [10] Charlet L, Chapron Y, Faller P, Kirsch R, Stone AT, Baveye PC. Neurodegenerative diseases and exposure to the environmental metals Mn, Pb, and hg. *Coordination Chemistry Reviews*. 2012;**256**:2147-2163
- [11] Holmes P, James KAF, Levy LS. Is low-level environmental mercury exposure of concern to human health? *Science of the Total Environment*. 2009;**408**:171-182
- [12] Lu H, Xiong L, Liu H, Yu M, Shen Z, Li F, You X. A highly selective and sensitive fluorescent turn-on sensor for Hg²⁺ and its application in live cell imaging. *Organic & Biomolecular Chemistry*. 2009;**7**:2554-2558
- [13] Liu W, Chen J, Xu L, Wu J, Xu H, Zhang H, Wang P. Reversible "off-on" fluorescent chemosensor for Hg²⁺ based on rhodamine derivative. *Spectrochimica Acta A*. 2012;**85**:38-42
- [14] Deng L, Zhou Z, Li J, Li T, Dong S. Fluorescent silver nanoclusters in hybridized DNA duplexes for the turn-on detection of Hg²⁺ ions. *Chemical Communications*. 2011;**47**: 11065-11067
- [15] Ma LJ, Li Y, Li L, Sun J, Tian C, Wu Y. A protein-supported fluorescent reagent for the highly-sensitive and selective detection of mercury ions in aqueous solution and live cells. *Chemical Communications*. 2008:6345-6347
- [16] Du J, Liu M, Lou X, Zhao T, Wang Z, Xue Y, Zhao J, Xu Y. Highly sensitive and selective chip-based fluorescent sensor for mercuric ion: Development and comparison of turn-on and turn-off systems. *Analytical Chemistry*. 2012;**84**:8060-8066
- [17] MacLean JL, Morishita K, Liu J. DNA stabilized silver nanoclusters for ratiometric and visual detection of Hg²⁺ its immobilization in hydrogels. *Biosensors & Bioelectronics*. 2013;**48**:82-86
- [18] Nolan EM, Lippard SJ. Tools and tactics for the optical detection of mercuric ion. *Chemical Reviews*. 2008;**108**:3443-3480
- [19] Ghaedi M, Fathi MR, Shokrollahi A, Shajarat F. Highly selective and sensitive preconcentration of mercury ion and determination by cold vapor atomic absorption spectroscopy. *Analytical Letters*. 2006;**39**:1171-1185
- [20] Turker AR, Cabuk D, Yalcinkaya O. Preconcentration, speciation spectrometry, and determination of mercury by solid phase extraction with cold vapor atomic absorption. *Analytical Letters*. 2013;**46**:1155-1170

- [21] Silva TS, Conte C, Santos JO, Simas ES, Freitas SC, Raices RLS, Quiterio SL. Spectrometric method for determination of inorganic contaminants (arsenic, cadmium, lead and mercury) in smooth weakfish fish. *LWT-Food Science and Technology*. 2017;**76**:87-94
- [22] Oehmen A, Vergel D, Fradinho J, Reis MAM, Crespo JG, Velizarov S. Mercury removal from water streams through the ion exchange membrane bioreactor concept. *Journal of Hazardous Materials*. 2014;**264**:65-70
- [23] Biester H, Schuhmacher P, Muëller G. Effectiveness of mossy tin filters to remove mercury from aqueous solution by Hg(II) reduction and Hg(0) amalgamation. *Water Research*. 2000;**34**:2031-2036
- [24] Huttenloch P, Roehl KE, Czurda K. Use of copper shavings to remove mercury from contaminated groundwater or wastewater by amalgamation. *Environmental Science & Technology*. 2003;**37**:4269-4273
- [25] Chojnacki A, Chojnacka K, Hoffman J, Gorecki H. The application of natural zeolites for mercury removal: From laboratory tests to industrial scale. *Minerals Engineering*. 2004;**17**:933-937
- [26] Evangelista SM, DeOliveira E, Castro GR, Zara LF, Prado AGS. Hexagonal mesoporous silica modified with 2-mercaptothiazoline for removing mercury from water solution. *Surface Science*. 2007;**601**:2194-2202
- [27] Lopes CB, Otero M, Coimbra J, Pereira E, Rocha J, Lin Z, Duarte A. Removal of low concentration Hg^{2+} from natural waters by microporous and layered titanosilicates. *Microporous and Mesoporous Materials*. 2007;**103**:325-332
- [28] Fábrega FM, Mansur MB. Liquid-liquid extraction of mercury(II) from hydrochloric acid solutions by aliquat 336. *Hydrometallurgy*. 2007;**87**:83-90
- [29] Pokhrel LR, Ettore N, Jacobs ZL, Zarr A, Weir MH, Scheuerman PR, Kanel SR, Dubey B. Novel carbon nanotube (CNT)-based ultrasensitive sensors for trace mercury(II) detection in water: A review. *Science of the Total Environment*. 2017;**574**:1379-1388
- [30] Liu Y, Wang X, Wu H. Reusable DNA-functionalized-graphene for ultrasensitive mercury (II) detection and removal. *Biosensors and Bioelectronics*. 2017;**87**:129-135
- [31] Wang N, Lin M, Dai H, Ma H. Functionalized gold nanoparticles/reduced graphene oxide nanocomposites for ultrasensitive electrochemical sensing of mercury ions based on thymine-mercury-thymine structure. *Biosensors and Bioelectronics*. 2016;**79**:320-326
- [32] Henriques B, Goncalves G, Emami N, Pereira E, Vila M, Marques PAAP. Optimized graphene oxide foam with enhanced performance and high selectivity for mercury removal from water. *Journal of Hazardous Materials*. 2016;**301**:453-461
- [33] Borthakur P, Darabdhara G, Das MR, Boukherroub R, Szunerits S. Solvothermal synthesis of CoS/reduced porous graphene oxide nanocomposite for selective colorimetric detection of Hg(II) ion in aqueous medium. *Sensors and Actuators B*. 2017;**244**:684-692

- [34] Saad A, Bakas I, Piquemal JY, Nowakc S, Abderrabba M, Chehimi MM. Mesoporous silica/polyacrylamide composite: Preparation by UV-graft photopolymerization, characterization and use as Hg(II) adsorbent. *Applied Surface Science*. 2016;**367**:181-189
- [35] Li R, Liu L, Yang F. Preparation of polyaniline/reduced graphene oxide nanocomposite and its application in adsorption of aqueous Hg(II). *Chemical Engineering Journal*. 2013;**229**:460-468
- [36] Palanisamy S, Thangavelu K, Chen SM, Velusamy V, Chang MH, Chen TW, Al-Hemaid FMA, Ali MA, Ramaraj SK. Synthesis and characterization of polypyrrole decorated graphene/ β -cyclodextrin composite for low level electrochemical detection of mercury (II) in water. *Sensors and Actuators B*. 2017;**243**:888-894
- [37] Kim JH, Noh JY, Hwang IH, Lee JJ, Kim C. A NBD-based selective colorimetric and fluorescent chemosensor for Hg²⁺. *Tetrahedron Letters*. 2013;**54**:4001-4005
- [38] Ozdemir M. A rhodamine-based colorimetric and fluorescent probe for dual sensing of Cu²⁺ and Hg²⁺ ions. *Journal of Photochemistry and Photobiology A: Chemistry*. 2016;**318**:7-13
- [39] Niu Q, Wu X, Zhang S, Li T, Cui Y, Li X. A highly selective and sensitive fluorescent sensor for the rapid detection of Hg²⁺ based on phenylamine-oligothiophene derivative. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2016;**153**:143-146
- [40] Lee S, Rao BA, Son YA. A highly selective fluorescent chemosensor for Hg²⁺ based on a squaraine-bis(rhodamine-B) derivative: Part II. *Sensors and Actuators B*. 2015;**210**:519-532
- [41] Dong Z, Tian X, Chen Y, Hou J, Guo Y, Sun J, Ma J. A highly selective fluorescent chemosensor for Hg²⁺ based on rhodamine B and its application as a molecular logic gate. *Dyes and Pigments*. 2013;**97**:324-329
- [42] Tang J, Huang Y, Zhang C, Liu H, Tang D. DNA-based electrochemical determination of mercury(II) by exploiting the catalytic formation of gold amalgam and of silver nanoparticles. *Microchimica Acta*. 2016;**183**:1805-1812
- [43] Brasca R, Onaindia MC, Goicoechea HC, Peña AM, Culzoni MJ. Highly selective and ultrasensitive turn-on luminescence chemosensor for mercury (II) determination based on the rhodamine 6G derivative FC1 and au nanoparticles. *Sensors*. 2016;**16**:1652
- [44] Le VS, Jeong JE, Huynh HT, Lee J, Woo HY. An ionic 1,4-bis(styryl)benzene-based fluorescent probe for mercury(II) detection in water via deprotection of the thioacetal group. *Sensors*. 2016;**16**:2082
- [45] Chen Y, Yao L, Deng Y, Pan D, Ogabiela E, Cao J, Adeloju SB, Chen W. Rapid and ultrasensitive colorimetric detection of mercury(II) by chemically initiated aggregation of gold nanoparticles. *Microchimica Acta*. 2015;**182**:2147-2154
- [46] Oskoei YM, Bagheri N, Hassanzadeh J. Ultrasensitive determination of mercury(II) using a chemiluminescence system composed of permanganate, rhodamine B and gold nanoprisms. *Microchimica Acta*. 2015;**182**:1635-1642

- [47] Tupys A, Kalemekiewicz J, Bazel Y, Zapala L, Dranka M, Ostapiuk Y, Tymoshuk O, Woznicka E. 1-[(5-Benzyl-1,3-thiazol-2-yl)diazonyl]naphthalene-2-ol: X-ray structure, spectroscopic characterization, dissociation studies and application in mercury(II) detection. *Journal of Molecular Structure*. 2017;**1127**:722-733
- [48] Fashi A, Yaftian MR, Zamani A. Electromembrane extraction-preconcentration followed by microvolume UV-Vis spectrophotometric determination of mercury in water and fish samples. *Food Chemistry*. 2017;**221**:714-720
- [49] Narayanana KB, Han SS. Highly selective and quantitative colorimetric detection of mercury(II) ions by carrageenan-functionalized Ag/AgCl nanoparticles. *Carbohydrate Polymers*. 2017;**160**:90-96
- [50] Cheng L, Wei B, He LL, Mao L, Zhang J, Ceng J, Kong D, Chen C, Cui H, Hong N, Fan H. "Off-on" switching electrochemiluminescence biosensor for mercury(II) detection based on molecular recognition technology. *Analytical Biochemistry*. 2017;**518**:46-52
- [51] Brent RN, Winesa H, Luther J, Irving N, Collins J, Drake BL. Validation of handheld X-ray fluorescence for in situ measurement of mercury in soils. *Journal of Environmental Chemical Engineering*. 2017;**5**:768-775
- [52] Sharma VV, Tonelli D, Guadagnini L, Gazzano M. Copper-cobalt hexacyanoferrate modified glassy carbon electrode for an indirect electrochemical determination of mercury. *Sensors and Actuators B*. 2017;**238**:9-15
- [53] Li XQ, Liang HQ, Cao Z, Xiao Q, Xiao ZL, Song LB, Chen D, Wang FL. Simple and rapid mercury ion selective electrode based on 1-undecanethiol assembled au substrate and its recognition mechanism. *Materials Science and Engineering C*. 2017;**72**:26-33
- [54] Brunton LL, Chbner BA, Knollmann BC. Goodman & Gilman's, *The Pharmacological Basic of Therapeutics*, 12th ed. USA: McGraw-Hill; 2011
- [55] Miller RD, Cohen NI, Eriksson LI, Fleisher LA, Wiener JP, Young WL. *Miller's Anesthesia*, 8th ed. Saunders, USA: An Imprint of Elsevier Inc.; 2015
- [56] Merino C, Junquera E, Barbero JJ, Aicart E. Effect of the presence of β -cyclodextrin on the solution behavior of procaine hydrochloride. *Spectroscopic and thermodynamic studies*. *Langmuir*. 2000;**16**:1557-1565
- [57] Lee AG. Interactions between anaesthetics amines and lipid mixtures. *Biochimica et Biophysica Acta*. 1976;**448**:34-44
- [58] Bartucci R, Mollica P, Sapia P, Sportelli L. Procain interaction with DPPC multilayers: An ESR spin label investigation. *Applied Magnetic Resonance*. 1998;**15**:181-195
- [59] Polasek M, Gas B. Determination of limiting ionic mobilities and dissociation constants of some local anaesthetics. *Journal of Chromatography*. 1992;**596**:265-270
- [60] El-Shahawi MS, Al Khateeb LA. Spectrofluorometric determination and chemical speciation of trace concentrations of tungsten species in water using the ion pairing reagent procaine hydrochloride. *Talanta*. 2012;**88**:587-592

- [61] Soni S, Pal A. Spectroscopic studies on host–guest interactions of α - and β -cyclodextrin with lidocaine hydrochloride and procaine hydrochloride. *Journal of Solution Chemistry*. 2016;**45**:665-674
- [62] El-Shahawi MS, Alwael H, Arafat A, Al-Sibaai AA, Bashammakh AS, Al-Harbi EA. Kinetics and thermodynamic characteristics of cadmium(II) sorption from water using procaine hydrochloride physically impregnated polyurethane foam. *Journal of Industrial and Engineering Chemistry*. 2015;**28**:147-152
- [63] El-Shahawi MS, Bashammakh AS, Orif MI, Alsibaai AA, Al-Harbi EA. Separation and determination of cadmium in water by foam column prior to inductively coupled plasma optical emission spectrometry. *Journal of Industrial and Engineering Chemistry*. 2014;**20**:308-314
- [64] El-Shahawia MS, Hamza A, Al-Sibaai AA, Al-Saidi HM. Fast and selective removal of trace concentrations of bismuth (III) from water onto procaine hydrochloride loaded polyurethane foams sorbent: Kinetics and thermodynamics of bismuth (III) study. *Chemical Engineering Journal*. 2011;**173**:29-35
- [65] Vogel AI. *Quantitative Inorganic Analysis*. 3rd ed. England: Longmans Group Ltd.; 1966
- [66] Sawyer DT, Heinemann WR, Beebe JM. *Chemistry Experiments for Instrumental Methods*. New York: John Wiley & Sons; 1984
- [67] Santoni G, Mura P, Pinzauti S, Lombardo E, Gratteri P. Simultaneous UV spectrophotometric determination of procaine hydrochloride and phenazone in an otic formulation. *International Journal of Pharmaceutics*. 1990;**64**:235-238
- [68] Xu LX, Shen YX, Wang HY, Jiang JG, Xiao Y. Spectrophotometric determination of procaine hydrochloride in pharmaceutical products using 1,2-naphthoquinone-4-sulfonic acid as the chromogenic reagent. *Spectrochimica Acta Part A*. 2003;**59**:3103-3110
- [69] Sekine T, Ishli T. Studies of the liquid-liquid partition systems. VII. The solvent extraction of mercury(II) chloride, bromide, iodide and thiocyanate with some organic solvent. *Bulletin of the Chemical Society of Japan*. 1970;**43**:2422-2429
- [70] Sharma RP, Singh A, Venugopalan P, Smolentsev AI, Gubanov AI. Stabilizing effects of large counter ion: Synthesis, characterization and single crystal X-ray structure determination of $[\text{Co}(\text{NH}_3)_6][\text{HgI}_4]\cdot\text{H}_2\text{O}$. *Journal of Molecular Structure*. 2010;**975**:1-4
- [71] Pourreza N, Behpour M. Column preconcentration of mercury as HgI_4^{2-} using methyl-triethylammonium chloride-naphthalene adsorbent with subsequent anodic stripping-differential pulse voltammetric determination. *Analytica Chimica Acta*. 2003;**481**:23-28
- [72] Liu J, Huang Y, Cai W, Li P, Li X, Li L, Lin S. Determination of trace mercury by solid substrate room temperature phosphorimetry based on an ionic associate $[(\text{Rhod.6G})_2]^{2+} \cdot [\text{HgI}_4]^{2-}$. *International Journal of Environmental Analytical Chemistry*. 2005;**85**:387-397
- [73] Yowloo AY, Lay YP, Kutty MG, Timpe O, Behrens M, Hamid SBA. Spectrophotometric determination of mercury with iodide and rhodamine B. *Sains Malaysiana*. 2012;**41**:213-218

- [74] Sun Y, Ma L, Wang HY, Tang B. Determination of procaine hydrochloride by fluorimetry. *Guang Pu Xue Yu Guang Pu Fen Xi*. 2002;**22**:637-640
- [75] Carretero AS, Blanco CC, Peinado SF, El-Bergmi R, Gutierrez AF. Fluorimetric determination of procaine in pharmaceutical preparations based on its reaction with fluorecamine. *Journal of Pharmaceutical and Biomedical Analysis*. 1999;**21**:969-974
- [76] Sanchez FG, Rubio ALR, Blanco CC, Lopez MH, Gomez JCM, Carnero C. Enhanced fluorimetric determination of procaine in pharmaceutical preparations by aqueous β -cyclodextrin inclusion and non-aqueous competitive action. *Analytica Chimica Acta*. 1988;**205**:139-147
- [77] Valeur B. *Molecular Fluorescence Principles and Applications*. Weinheim, Federal Republic of Germany: Wiley-VCH Verlag GmbH; 2002
- [78] Miller JC, Miller JN. *Statistics for Analytical Chemistry*. 6th ed. New York.: Ellis-Howood; 2010
- [79] Skoog DA, West DM, Holler FJ, Crouch SR. *Fundamental of Analytical Chemistry*. 9th ed. Belmont, USA: Cengage Learning; 2014
- [80] Maafi M, Laassis B, Aaron JJ. Photochemically induced fluorescence investigation of a β -cyclodextrin: Azure A inclusion complex and determination of analytical parameters. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry*. 1995;**22**:235-247
- [81] Abdel-Shafi AA, Al-Shihry SS. Fluorescence enhancement of 1-naphthol-5-sulfonate by forming inclusion complex with β -cyclodextrin in aqueous solution. *Spectrochimica Acta Part A*. 2009;**72**:533-537
- [82] Al-Shihry SS. Spectroscopic studies of inclusion complexes of 1-naphthol-4-sulfonate with β -cyclodextrin in aqueous solution. *Spectrochimica Acta Part A*. 2005;**61**:2439-2443
- [83] Kiwan AM, El-Shahawi MS, Aldhaheri SM, Saleh MH. Sensitive detection and semi-quantitative determination of mercury(II) and lead(II) in aqueous media using polyurethane foam immobilized 1,5-di-(2-fluorophenyl)-3-mercaptopformazan. *Talanta*. 1997;**45**:203-211
- [84] Fernandez E, Vidal L, Costa-Garcia A, Canals A. Mercury determination in urine samples by nanostructured screen-printed carbon electrodes after vortex-assisted ionic liquid dispersive liquid-liquid microextraction. *Analytica Chimica Acta*. 2016;**915**:49-55