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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

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 µg 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 $[(PQ^+)_2.(HgI_4)^{2-}]$ of $[HgI_4]^{2-}$ with the proposed ion pairing reagent [60–64].
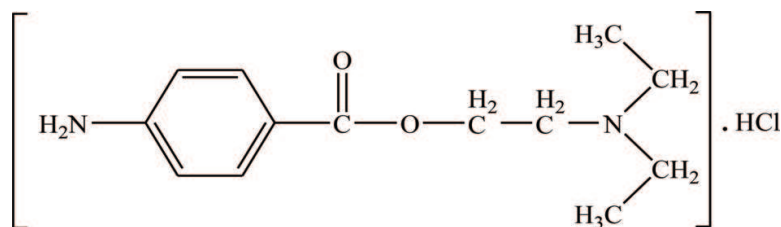
Figure 1. Chemical structure of procaine hydrochloride.

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 μM) 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 μM) 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 × 10 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 × 10^{-5} M) was transferred to a series of glass test tubes (10.0 mL) followed by addition of 100 μL 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 $PQ^+.Cl^-$ 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 $\lambda_{ex/em}$ = 268/333 nm *versus* a reagent blank at room temperature. The quenched fluorescence in signal intensity (ΔF) of $PQ^+.Cl^-$ by mercury(II) was computed employing the following equation:

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

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 μg 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 μM) 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.
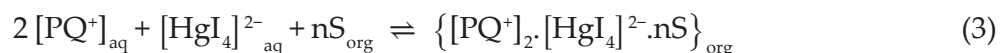
2.4. Analytical applications

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 μm 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(1I) 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 → π* 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]:

$$Hg^{2+} + 4I^- \rightleftharpoons [HgI_4]^{2-} \tag{2}$$

$$2\,[PQ^+]_{aq} + [HgI_4]^{2-}{}_{aq} + nS_{org} \rightleftharpoons \left\{[PQ^+]_2.[HgI_4]^{2-}.nS\right\}_{org} \tag{3}$$

As shown in **Figure 3**, the solution of reagent blank $PQ^+.Cl^-$ with concentration of 5.0 μM 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
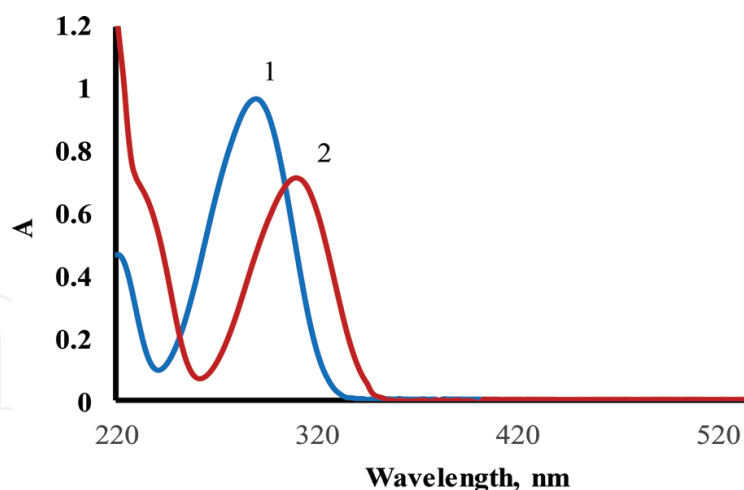
Figure 2. Electronic spectra of the PQ⁺.Cl⁻ (4 × 10⁻⁵ M) (1) and its ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ in dichloromethane (2). Condition: Mercury (1 × 10⁻⁵ M).

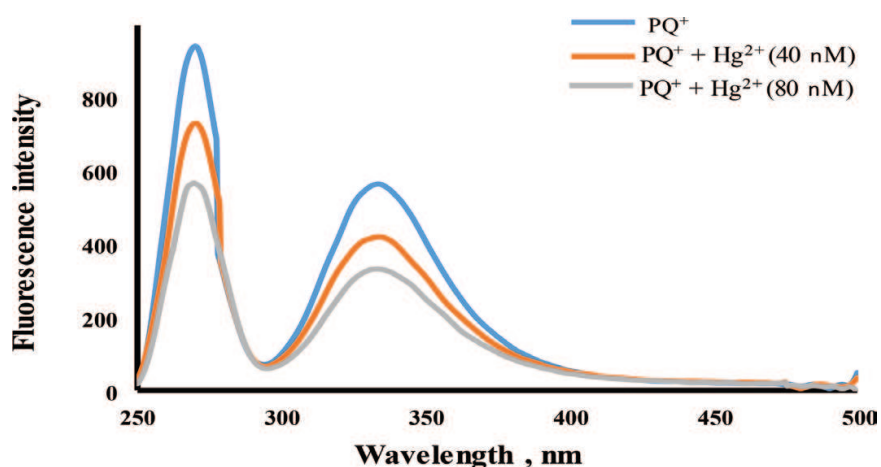**Figure 3.** Fluorescence spectra of PQ⁺.Cl⁻ (5.0 μM) and its ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$ with mercury(II) ions (40, 80 nM).

complex $[(PQ^+)_2.(HgI_4)^{2-}]$ (**Figure 3**) between PQ⁺.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
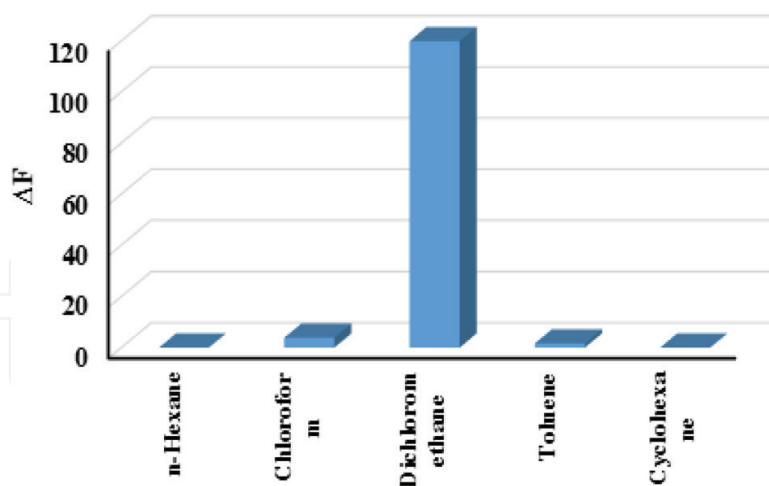
Figure 4. Influence of type extraction solvent on the fluorescence quenching of $PQ^+.Cl^-$ (5.0 μM) reagent by $K_2[HgI_4]$ (80 nM).

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 ≈ 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 ≈ 9–10 [62, 63]. Thus, in the next work, the solution media were adopted at pH ≈ 10 due to the ease in formation of the ternary ion associate complex $[(PQ^+)_2.(HgI_4)^{2-}]$.
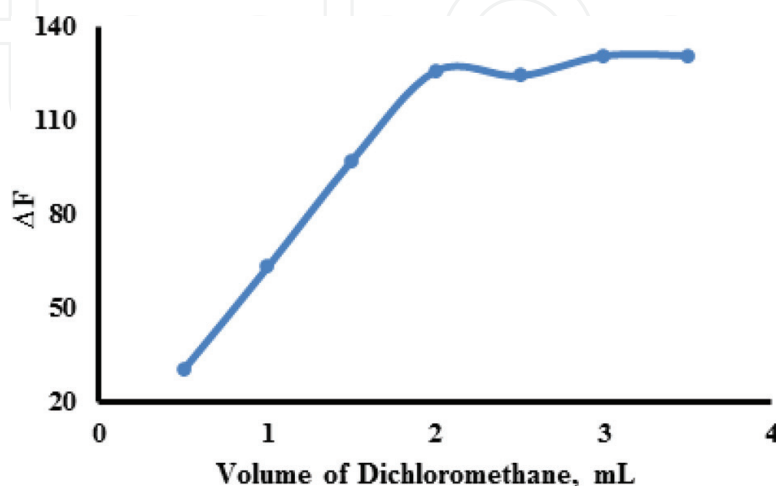

Figure 5. Influence of dichloromethane volume on the fluorescence quenching of $PQ^+.Cl^-$ (5.0 μM) reagent by mercury (80 nM).

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 µgL^{-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
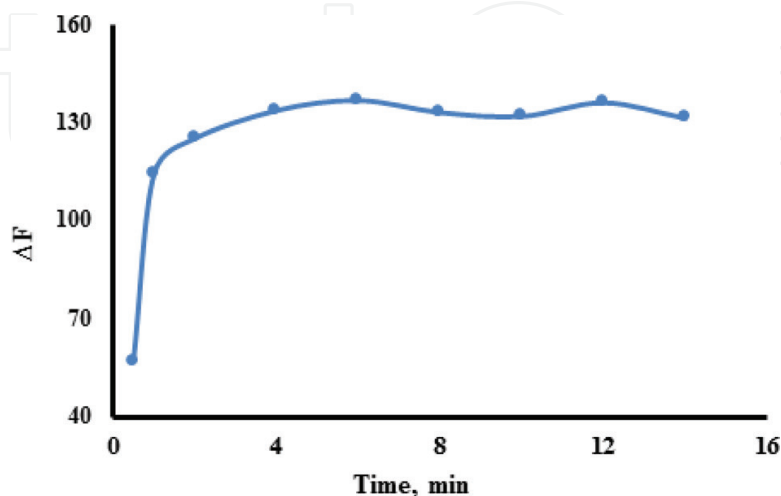

Figure 6. Plot of time *vs.* the fluorescence quenching of $PQ^+.Cl^-$ (5.0 µM) reagent by mercury (80 nM).
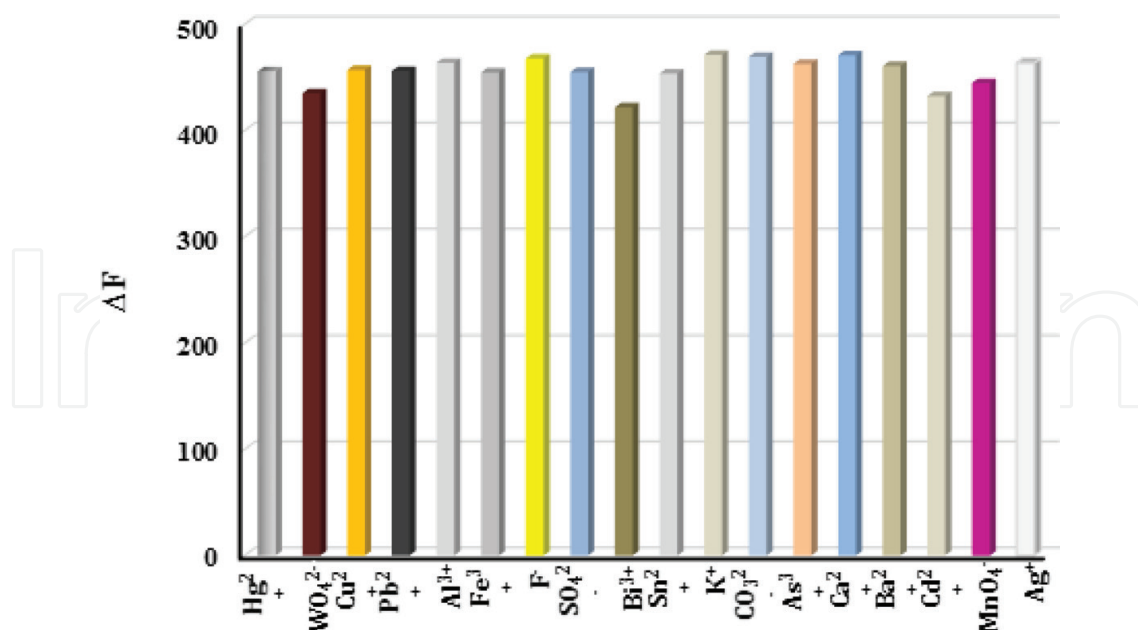
Figure 7. The values of quenching fluorescence intensity of $PQ^+.Cl^-$ (5.0 μgL^{-1}) with addition of Hg(II) (2.0 μgL^{-1}) in the presence of different metal ions (500 μgL^{-1}) in KI (0.1% w/v).

$PQ^+.Cl^-$ possess good selectivity in probing mercury(II). As shown in **Figure 7**, the signal intensity in the presence of 2.0 $\mu g\ L^{-1}$ mercury(II) mixed with 500 $\mu g\ L^{-1}$ of other metal ions in KI (0.1% w/v) was found similar to mercury(II) alone at 2.0 $\mu g\ L^{-1}$ 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:

$$\Delta F = 1.6354\ [C]\ (nM)–3.3971 \tag{4}$$

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
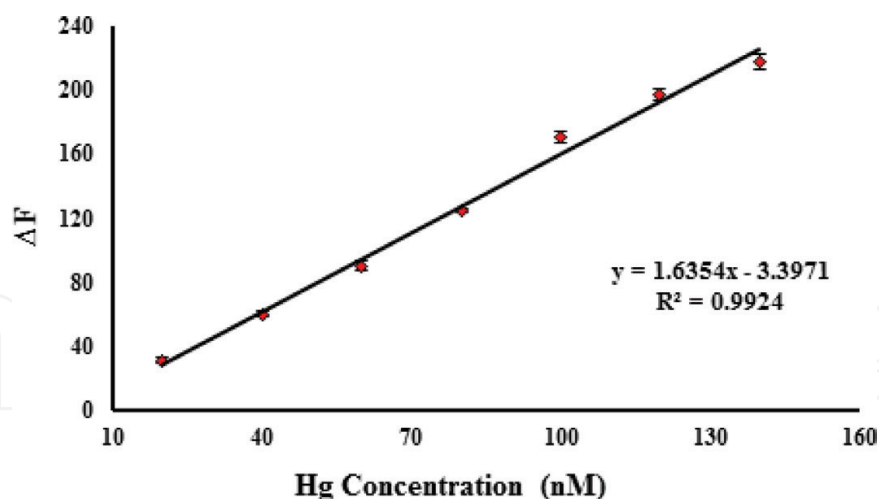
Figure 8. Calibration plot of ternary ion associate complex [(PQ⁺)₂.(HgI₄)²⁻]. Conditions: Mercury(II) (20–140 nM), PQ⁺ (5.0 μM) and KI (0.1% w/v) pH ≈ 10 at $\lambda_{ex/em}$ = 268/333 nm.

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.(HgI₄)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 (0.3–1.0 μg L^{-1}) of quencher (mercury(II) ions) are shown in **Figure 9**. The fluorescence intensity of PQ$^+$.Cl$^-$ decreases regularly with increasing quencher concentration. The Stern-Volmer (K$_{SV}$) constant was calculated by employing the equation:

$$F_0/F \; = \; 1 + K_{SV} \, [Q] \qquad (5)$$

where F$_0$ and F are the fluorescence signals in the absence and presence of [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 PQ$^+$. Cl$^-$ *versus* [Hg^{2+}] were found equal to 1.87 × 10^6 L g^{-1} mol^{-1} and 0.9909, respectively.

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

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

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

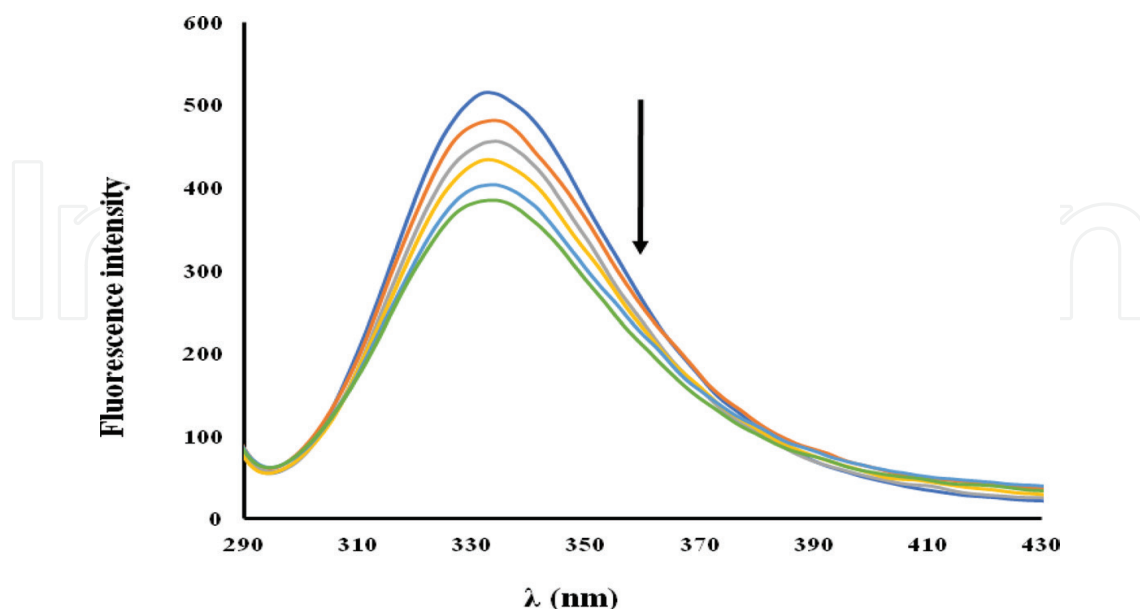**Figure 9.** Fluorescence quenching spectra of PQ$^+$.Cl$^-$ (5.0 μM) in the presence of various concentrations (20–80 nM) of the mercury(II) ions. Conditions: KI (0.1% w/v) pH ≈ 10, at λ$_{ex/em}$ = 268/333 nm.

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

[†]Average ± 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 $[(PQ^+)_2.(HgI_4)^{2-}]$ between $[HgI_4]^{2-}$ 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.

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