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Cloud point extraction and spectrophotometric determination of mercury species at trace levels in environmental samples

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ABSTRACT

A new micelle-mediated separation and preconcentration method was developed for ultra-trace quantities of mercury ions prior to spectrophotometric determination. The method is based on cloud point extraction (CPE) of Hg(II) ions with polyethylene glycol *tert*-octylphenyl ether (Triton X-114) in the presence of chelating agents such as 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-thiazolylazo) resorcinol (TAR). Hg(II) ions react with both PAN and TAR in a surfactant solution yielding a hydrophobic complex at pH 9.0 and 8.0, respectively. The phase separation was accomplished by centrifugation for 5 min at 3500 rpm.

The calibration graphs obtained from Hg(II)–PAN and Hg(II)–TAR complexes were linear in the concentration ranges of 10–1000 $\mu g\,L^{-1}$ and 50–2500 $\mu g\,L^{-1}$ with detection limits of 1.65 and 14.5 $\mu g\,L^{-1}$, respectively. The relative standard deviations (RSDs) were 1.85% and 2.35% in determinations of 25 and 250 $\mu g\,L^{-1}$ Hg(II), respectively. The interference effect of several ions were studied and seen commonly present ions in water samples had no significantly effect on determination of Hg(II). The developed methods were successfully applied to determine mercury concentrations in environmental water samples. The accuracy and validity of the proposed methods were tested by means of five replicate analyses of the certified standard materials such as QC Metal LL3 (VWR, drinking water) and IAEA W-4 (NIST, simulated fresh water).

1. Introduction

Mercury is a serious environmental pollutant because of its toxic effects on all living organisms [1]. Mercury and its compounds cause serious diseases such as leukemia [2]. Mercury compounds can be present as a result of anthropogenic activities in various environmental samples [3]. They are usually present in natural waters at trace levels [3,4]. The lakes, rivers in vicinity of the industrial areas are the important indicators for mercury pollution. So, it needs to develop new, selective, effective, cheap methods for determination of mercury [5].

A serious problem in the determination of mercury is related to low concentrations of target species. The main species of mercury in natural waters are inorganic mercury (Hg_2^{2+}, Hg^{2+}) and methyl mercury (CH_3Hg^+). Recent reports estimate that total mercury concentration is in the range of 0.2–100 $ng\,L^{-1}$ and methyl mercury concentrations are lower (ca. 0.05 $ng\,L^{-1}$) in natural waters [6].

Numerous analytical and sophisticated techniques such as inductively coupled plasma mass spectrometry (ICP-MS) [7,8], inductively coupled plasma atomic emission spectrometry (ICP-AES) [9,10], cold vapor atomic absorption spectrometry (CV-AAS) [11–13], neutron activation analysis (NAA) [14], X-ray fluorescence spectrometry (XRF) [15], atomic fluorescence spectrometry (AFS) [16,17], and spectrophotometry [18–21] have been developed to determine Hg(II) at trace level. Each of the mentioned techniques has its own merits, but each method also offers some problems such as poor reproducibility and limited sample adaptability. ICP-AES and ICP-MS are useful for trace determination without any preconcentration. However, these instruments are very expensive to purchase and operate. Moreover, these techniques have some inherent interference [7,10]. CV-AAS is a suitable and widely used technique for accurate determination of mercury due to its simplicity. But, its usage is limited because of a narrow linear range and spectral interference from volatile species [12,22,23]. Therefore, this technique is not directly applicable to environmental and biological samples in view of low analyte contents and it requires preconcentration steps to enhance the sensitivity. A number of photometric reagents such as 1-(2-pyridylazo)-2-naphthol (PAN), 4-(2-pyridylazo) resorcinol (PAR), crystal violet, triphenyltetrazolium chloride, triphenylphosphine oxide, and diphenylcarbazone have been used for spectrophotometric determination of Hg(II). But, dithizone, which forms a water-insoluble complex, is the most

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commonly used [18–21,23]. The formed complex is extracted either in CCl_4 or $CHCl_3$ before photometric determination [24,25].

Drinking water is one of the routes of mercury entrance into the human body is drinking water. Hence, mercury determination in this type of sample is very important. However, mercury concentration in drinking water is lower than classical method's detection limit. Therefore, it is usually need to apply a preconcentration step.

The use of CPE [26] offers an alternative to conventional extraction systems. Aqueous solutions are used in the CPE method instead of toxic and flammable organic solvents. In addition, CPE offers higher recovery efficiency and a large pre-concentration factor.

The CPE method has been used to pre-concentrate mercury ions after the formation of sparingly water-soluble complexes, as a prior step to their determination [27–37]. In one method [36], mercury was pre-concentrated by CPE prior to ICP-OES coupled to FI-CVAAS. The mercury ions were extracted as mercury-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol [Hg(II)-(5-Br-PADAP)] complex in the presence of non-ionic surfactant polyethyleneglycolmono-*p*-nonylphenylether (Ponpe 7.5) at pH 9.2. The calibration graph was linear from detection limits up to 50 $\mu g\,L^{-1}$, and the detection limit was 0.004 $\mu g\,L^{-1}$. In second method [37], mercury was pre-concentrated by CPE method as a mercury-dithizone complex in Triton X-100 micellar media. The calibration was linear from 0.05 to 0.50 $\mu g\,mL^{-1}$ and the limit of detection was 0.014 $\mu g\,mL^{-1}$.

In the present study, two detection methods were developed for trace mercury ions based on the absorbance measurements of the hydrophobic complexes against the reagent blank. The absorbance values measured at 554 and 389 nm obey to Beer law. The methods were applied successfully to the determination of Hg(II) and total Hg at trace levels after CPE in environmental water samples, industrial waste water as well as certified standard water samples. The advantages of these methods are simplicity, selectivity, sensitivity, cheapness, wide linear range, and applicability to real samples. The used instrumentation for determination of trace mercury ions is expensive and they cannot be provided in every basic analysis laboratory. The proposed methods use only a conventional spectrophotometer after a simple CPE procedure. All essential equipment for the proposed methods can be provided in almost every laboratory. Determination of trace mercury ions in real samples can be achieved one of the methods or both according to method's linear range.

2. Experimental

2.1. Instrumentation

A spectrophotometer (Shimadzu Model UV-1800, Japan) equipped with a 1 cm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm and a bandwidth of 2 nm in the wavelength range of 190–1100 nm. A pH meter with a glass-calomel electrode (Selecta, Spain) was used to measure the pH values. A centrifuge (Hettich Universal) was used to accelerate the phase separation. A thermostated water bath (Nuve NT 120, Turkey) was used for the CPE experiments.

2.2. Reagents

Ultra-pure water with a resistivity of 18.2 MΩ cm was used during trace analysis provided by Milli-Q water purification system. All containers (glassware, PTFE bottles) were treated firstly with diluted HNO_3 solution and then with diluted HCl solution, finally they were rinsed with deionized water prior to experiments. Stock solutions of Hg(II) and Hg(I) (1000 $\mu g\,mL^{-1}$) were prepared by dissolving appropriate amounts of their nitrate salts in deionized water. Stock solutions of 2.0×10^{-3} mol L^{-1}, 1-(2-pyridylazo)-2-naphthol (PAN) and 2.85×10^{-4} mol L^{-1}, 4-(2-tiazoyliazo)-resorcinol (TAR) (Sigma, St. Louis, MO, USA) were prepared by dissolving the reagents in ethanol (Merck, Darmstadt, Germany) and diluting with water. Solution of 5% (w/v) Triton X-114 (Sigma) was prepared by dissolving 5 g of surfactant in 100 mL of deionized water. A 0.04 mol L^{-1} of Britton-Robinson (BR) buffers were used to keep the desired pH values. This buffer consists of a mixture of 0.04 mol L^{-1} H_3BO_3 (Merck), 0.04 mol L^{-1} H_3PO_4 (Merck) and 0.04 mol L^{-1} CH_3COOH (Merck) that has been titrated to the desired pH with 0.2 mol L^{-1} NaOH.

2.3. The CPE procedure

Aliquots of sample or pretreated-sample containing Hg(II) in the range of 10–1000 $\mu g\,L^{-1}$ for PAN and in the range of 50–2500 $\mu g\,L^{-1}$ for TAR were transferred into centrifuge tubes (50 mL in capacity). In the PAN method, samples were added 2.0 mL of pH 9.0 BR buffer, 0.4 mL of 5.0% (w/v) Triton X-114 and 0.5 mL of 2.0×10^{-3} mol L^{-1} PAN. In the TAR method, samples were added 1.5 mL of pH 8.0 BR buffer, 0.5 mL of 5.0% (w/v) Triton X-114 and 0.7 mL of 2.85×10^{-4} mol L^{-1} TAR. Then, the solutions were mixed and kept in a thermostatic water bath for 10 min at 50 °C. The phase separation was accelerated by centrifuging at 3500 rpm for 5 min. The mixtures were then cooled in an ice-bath for 5 min in order to increase the viscosity of the surfactant-rich phase and facilitate the removal of the aqueous phase. Then, the aqueous phase was easily separated from surfactant-rich phase by inverting the tube. 1.5 mL of ethanol solution was added to the surfactant-rich phase to reduce its viscosity prior to spectrophotometric detection at 554 nm for PAN and 389 nm for TAR, respectively. Finally, the mercury concentrations were determined by using either the directly calibration curve obtained by spectrophotometer or standard addition curve approach.

2.4. Analysis of water samples

Prior to preconcentration procedure, all water samples were filtered by 0.45 μm pore size membrane filters and they were stored at 4 °C. 500 mL of water samples were concentrated by evaporation to a final volume of 50 mL. Then, the proposed methods were applied to water samples. Standard addition method was used in order to calculate recovery values and check correctness of results.

The water samples including lake water, river water, dental wastewater, and industrial wastewaters were similarly preconcentrated by evaporation. The CPE procedure was applied for determinations of Hg(II) and total Hg contents before and after oxidation with $KMnO_4$ in acidic medium. The standard addition method and calibration method was used for analysis of water samples. The certified water samples such as QC Metal LL3 and NIST, IAEA/W-4 which are used to verify the validity of the methods, were directly analyzed by using standard addition approach without any pretreatment.

3. Results and discussion

Fig. 1(a) and (b) shows the absorption spectra for the Hg(II) complexes, PAN and TAR in surfactant-rich phase against reagent blank with increasing mercury concentration at three different mercury levels.

3.1. Effect of pH

The pH is a critical factor affecting both the reaction between metal ions and ligand molecules, and the metallic complex

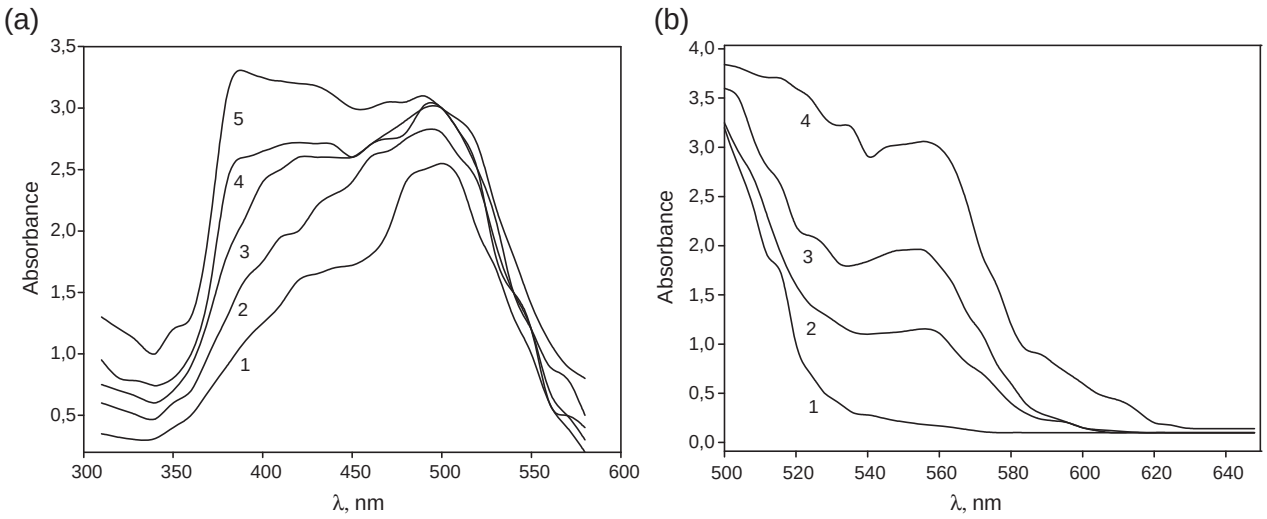

Fig. 1. (a and b) Absorption spectra of (a) 3.99×10^{-6} mol L^{-1} of TAR with increasing Hg(II) concentration at levels of 150, 500, 1000 and 1500 μg L^{-1} at pH 8.0 against reagent blank at 389 nm and (b) 2.00×10^{-5} mol L^{-1} of PAN with increasing Hg(II) concentration at levels of 100, 250 and 500 μg L^{-1} at pH 9.0 against reagent blank at 554 nm after preconcentration with CPE under the optimized reagent conditions.

extractability into the surfactant-rich phase. In this context, the complexation reaction of PAN (available in forms of LH_2^+, LH and L^- depending on pH of the environment) with Hg^{2+} ions are strongly dependent on pH of the solution because PAN is an organic ampholyte which in acidic medium can attract a proton to its pyridine nitrogen atom while in basic medium its o-hydroxy group can dissociate with acidity constants of pK_{a1}: 3.55 and pK_{a2}: 4.27 [38]. TAR is a well-known chelating reagent, which is used as an indicator in acid–base titrations [39,40]. The TAR has three acidity constants. Two of them (pK_{a1}: 5.98 and pK_{a2}: 9.70) are due to the two ionizable OH groups and the third one is due to the dissociation of the protonated species (–N=NH^+–) at pH lower than 1.0 [41].

In order to determine the optimum pH, NH_3/NH_4Cl, $H_2PO_4^-/HPO_4^{2-}$, borate and Britton-Robinson (BR) buffer systems in range of 2.0–12.0 were used independently. The best analytical signal was obtained with 0.04 M of BR buffer system. As can be seen in Fig. 2(a), the maximum absorbance was obtained at pH 9.0 for PAN and pH 8.0 for TAR complexes, respectively. The effect of buffer concentration on the analytical signal was studied in the range of 0–5 mL (in final volume of 50.0 mL), and as can be seen in Fig. 2(b), the best analytical signal was obtained with buffer volume of 1.5 mL for PAN and 2.0 mL for TAR, respectively.

3.2. Effect of complexing agent concentration

The effect of concentrations of PAN and TAR on analytical response are shown in Fig. 2(c) and (d). As it can be seen for Hg–PAN complex, absorbance increases up to a known concentration of PAN, reaching a plateau in the range of 2.0–3.2 $\times 10^{-5}$ mol L^{-1}, where the reaction is completed. So, a concentration of 2.0×10^{-5} mol L^{-1} of PAN was chosen as the optimal value. Similarly, the signal increases with TAR concentration in the range of 6.0–7.5 $\times 10^{-6}$ mol L^{-1} and reaches a maximum value in 1.2×10^{-5} mol L^{-1}, which is considered as complete extraction. Therefore, a concentration of 1.2×10^{-5} mol L^{-1} of TAR was chosen as the optimal concentration.

Job's Method was used in order to define stoichiometry of Hg(II)–PAN and –TAR complexes. Iso molar series at concentrations of 2.49×10^{-4} mol L^{-1} for Hg(II), PAN and TAR were prepared. The absorbance of mixtures was measured at 554 for PAN and 389 nm for TAR. The results showed that each of the curves reaches a single maximum value in same molar ratio (1:2). This proves that a single complex compound is formed in the system, having a $Hg(PAN)_2$ and $Hg(TAR)_2$ composition.

3.3. Effect of nonionic surfactant concentration

The Triton X-114 and Ponpe 7.5 were chosen as nonionic surfactant because of their commercial availability in a high-purified homogenous form, low toxicological properties and cost. Additionally, the cloud point temperatures of these surfactants permit their use in the extraction and preconcentration of a large number of molecules. According to this investigation, Triton X-114 is more suitable than Ponpe 7.5. The variation of absorbance of Hg(II)–PAN and Hg–TAR complexes are shown as a function of the concentration of Triton X-114 in Fig. 2(e) and (f). A concentration of 0.04% (w/v) Triton X-114 for PAN and 0.05% (w/v) Triton X-114 for TAR was chosen as optimum concentration. At lower concentrations, the extraction efficiency of complexes is low probably because of the inadequacy of the assemblies to entrap the hydrophobic complex quantitatively. Above these concentrations, the analytical signal gradually decreases, and remains constant at the range of 0.04–0.07% especially for PAN and then decreases again. This decrease in analytical signal may be due to the increase of the surfactant volume, deteriorating the absorbance signal. Therefore, a concentration of 0.04% Triton X-114 for PAN and 0.05% Triton X-114 for TAR complexes was employed in all further studies.

3.4. Effect of the incubation temperature and time

Two important parameters in CPE are incubation time and equilibration temperature. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of phases. The dependence of extraction efficiency upon equilibrium temperature and time was studied over ranges of 20–70 °C and 5–30 min, respectively. The results showed that an equilibrium temperature of 45 °C is appropriate for both PAN and TAR. It also is enough 10 min as incubation time for both PAN and TAR in order to achieve quantitative extraction.

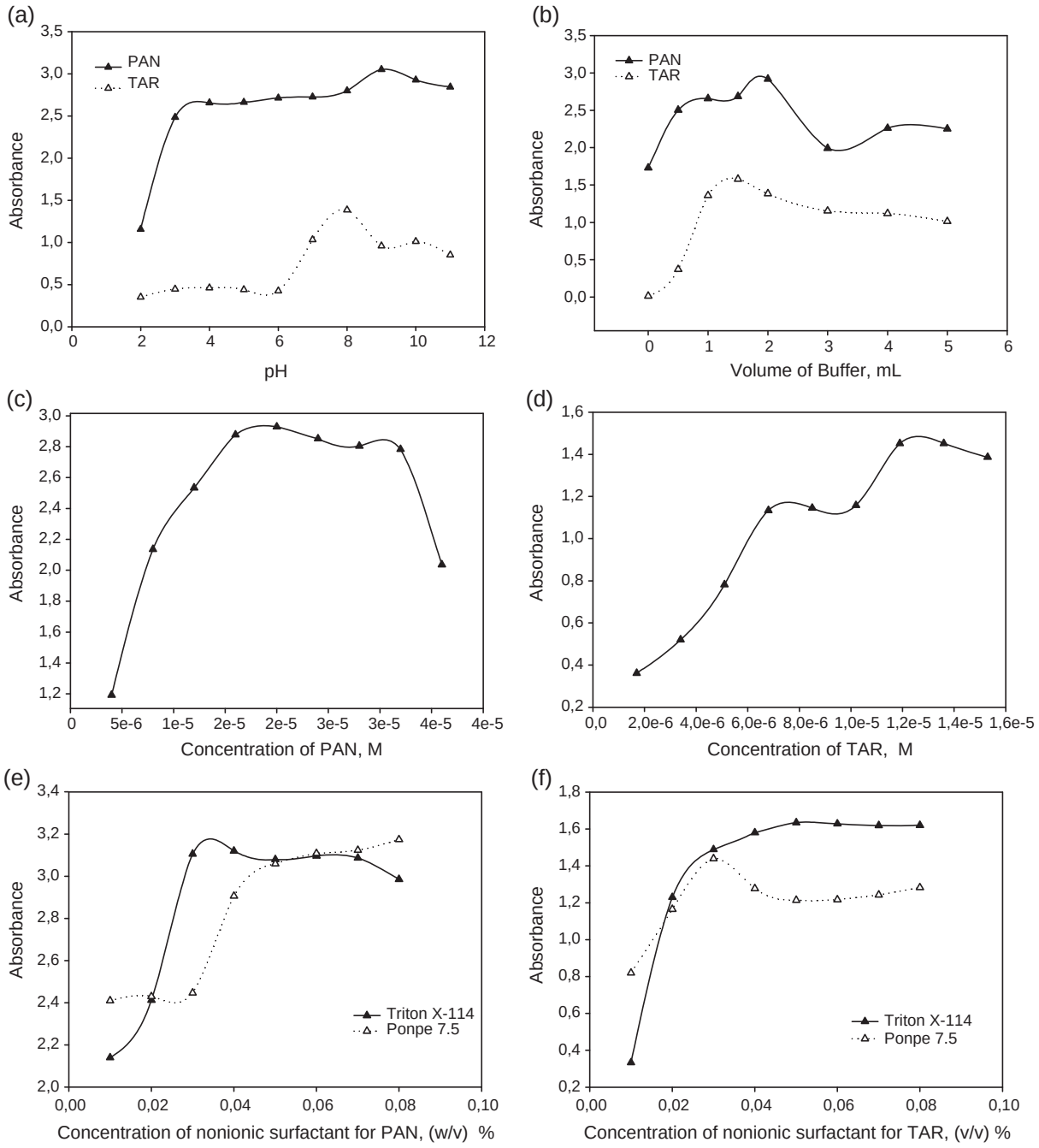

Fig. 2. (a) Effect of pH on the CPE, (b) effect of the buffer volume, (c) effect of PAN concentration, (d) effect of TAR concentration, (e) effect of nonionic surfactant concentration on absorbance of the Hg(II)–PAN and (f) effect of nonionic surfactant concentration on absorbance of the Hg(II)–TAR. Conditions: 250 μg L^{-1} of Hg(II) and (a) 0.5 mL of 2.0×10^{-3} M PAN, 0.4 mL of 5% (w/v) Triton X-114. (b) 0.7 mL of 2.85×10^{-4} M TAR, 0.5 mL of 5% (w/v) Triton X-114.

3.5. Effects of NaCl concentration

In the extraction methods, the solubility of many analytes in aqueous solutions decreases with increasing ionic strength due to the salting out effect [42]. In order to study the effect of the addition of electrolyte on micellar solutions of mercury ions, NaCl solution was investigated as electrolyte in the concentration range from 0.005 to 0.070 mol L^{-1}. The results show that addition of NaCl does not have an important effect on CPE experiments until concentration of 0.04 mol L^{-1}. In higher concentrations above 0.04 mol L^{-1}, the absorbance of complexes began decreasing specially for PAN.

3.6. Interference studies

In view of the high selectivity provided by spectrophotometry at the characteristic absorption wavelengths of 554 and 389 nm, the only interference may be attributed to the preconcentration step. In order to perform this study, interfering ions in different concentrations were added to a solution containing 250 μg L^{-1} of Hg(II) and were applied proposed methods. The tolerance limits were determined for a maximum error of ±5% and the results are given in Table 1. These results demonstrate that the common coexisting ions did not have significant effect on the

Table 1
Tolerance limit of interfering ions in determination of 250 μg L^{-1} Hg(II) ion using micellar spectrophotometric detection after preconcentration with CPE under the optimized conditions.

Ions	Interference/analyte ratio	
	In presence of PAN at 554 nm	In presence of TAR at 389 nm
H_3BO_3, HCO_3^-, CO_3^{2-}, F^-, Cl^-, Br^-, I^-, NO_3^- and SO_4^{2-}	1250–2500	1500–2500
NH_4^+, Na^+ and K^+	500–1000	500–1250
Mg^{2+}, Ca^{2+}, Sr^{2+} and Al^{3+}	100–250	150–350
CN^- and SCN^-	75–100	125–150
NO_2^-, Co^{2+} and Zn^{2+}	25–50	35–75
Mn^{2+}, Cd^{2+}, SO_3^{2-}, Hg_2^{2+} and Fe^{3+}	15–25	20–35
Ni^{2+}, Co^{2+}, Fe^{2+}, Pb^{2+}, Sb^{3+} and Cr^{3+}	5–15	10–30
Cu^{2+} and Bi^{3+}	2–5 (25–50)[a]	1–10 (35–75)[a]

[a] Tolerance limits in the presence of 0.1 mL of 0.05 M thiourea and 0.2 mL of 0.05 M $Na_4P_2O_7$ as masking agents.

determination of the analyte ions. Both PAN and TAR methods were observed to be fairly selective for Hg(II) ions at pH 9.0 and 8.0, respectively. Cu(II), Sb(III) and Bi(III) ions were found to interfere at tolerance limits ranging from 1 to 10. Interferences by Cu(II) and Bi(III) ions depended on chelating agent concentration. Their tolerance limits were increased 25–75 folds by using 0.2 mL of 0.05 mol L^{-1} thiourea and $Na_4P_2O_7$. Moreover, the concentrations of these ions are usually very low in most water samples and thus they have no interference in the extraction and determination mercury. Since commonly present ions in water samples did not affect significantly the recovery of Hg(II), the methods can therefore be applied to determination of Hg(II) species in environmental water samples.

3.7. Analytical characteristics

Table 2 summarizes the analytical characteristics such as regression equation, linear range, and limits of detection and quantification, reproducibility and preconcentration factors. The limits of detection and quantification were 1.65 and 5.36 μg L^{-1} for PAN and 14.50 and 47.15 μg L^{-1} for TAR. These concentrations intervals are appropriate for measurement mercury ion concentrations in water samples according to USEPA standards. Because, the amount of mercury in 50 mL of sample solution is measured after preconcentration by CPE in a final volume of 1.5 mL, the preconcentration factor was calculated by a factor of 33.3. The RSD for five replicate measurements of 25 μg L^{-1} of mercury with PAN was 2.75% while it is 2.65% for 250 μg L^{-1} of mercury with TAR.

Table 2
Analytical characteristics of the proposed method with and without CPE.

Parameters	With PAN at 554 nm	With TAR at 389 nm
Linear range (μg L^{-1})	10–1000	50–2500
Slope	0.0035	0.00113
Intercept	0.3242	0.417
Correlation coefficient (r^2)	0.9904	0.9883
Recovery% (n: 3)	98.7–103.5	97.8–102.7
RSD (%) (25 and 250 μg L^{-1}, n: 5)	2.75	2.65
LOD (μg L^{-1})	1.65	14.50
LOQ (μg L^{-1})	5.54	47.15
[a]Preconcentration factor	33.3	33.3

[a] Preconcentration factor is defined as the ratio of the initial solution volume to the volume of surfactant rich phase.

3.8. Determination of mercury species in water samples

The accuracy and validity of the proposed method was checked by applying the determination of mercury concentration in various water samples collected from environmental matrices. The wastewater samples were collected from the chrome plating industry, textile dyeing industry, and dental hospital wastewater. Then, they were analyzed for the presence of mercury by the proposed methods. The results are shown in Table 3. Recovery studies were also carried out after it was spiked to samples known concentrations of mercury at levels of 100 and 300 μg L^{-1}. The proposed methods were also applied to determination of mercury in potable water samples like river, lake and tap waters. The accuracy of the methods was statistically tested by comparing the obtained results with independent spectrophotometric PAN and TAR methods based on preconcentration with CPE. In addition, the accuracy was verified by recovery studies.

The methods were applied to six different water samples. The results from the PAN and TAR methods are in good agreement based on standard addition curve approach. The accuracy was verified by the Student's-test. According to this test, the calculated t values (in the range of 0.086–0.482) are less than the theoretical value (2.78, n: 5) at a confidence level of % 95. In addition, the statistical F-test was used for comparing the precision of the spectrophotometric PAN method with those of the TAR method. The $F_{4,4}$-test value at 95% confidence level did not exceed the theoretical value (6.39, n: 8) with a value ranging from 1.08 to 5.90 for F-test, indicating no significant difference between the performance of methods.

Additionally, the proposed methods were applied to a standard reference material (QC METAL LL3, mercury in water) with a mercury content of 6.48 ± 0.51 μg L^{-1}. It was analyzed by using the proposed methods. Similarly, the proposed method was also applied to another standard reference material (NIST, IAEA/W-4 simulated fresh water) with a mercury content of 2.5 ± 0.1 μg L^{-1} for total Hg. The results can be seen in Table 3. There was no significant difference between results obtained from proposed methods.

3.9. Determination of Hg(II) and Hg(I) species in mixtures

Suitable aliquots of Hg(II) + Hg(I) mixtures (preferably at ratios; 1:1, 1:5, 1:10 and 1:15) were taken in a 25 mL of conical flask. A few drops of 1.0 mol L^{-1} H_2SO_4 and 1.5 mL of 1% (w/v) $KMnO_4$ solution were added to oxidize Hg(I) ions. Then, the diluted mixture with 5 mL of water was heated in water bath for 15 min. After it was cooled to room temperature, 3 drops of 1% (w/v) NaN_3 solution was added to mixture. The reaction mixture was neutralized with diluted NH_4OH and transferred into a 50 mL volumetric flask. Then, the mixtures were analyzed by both of proposed methods. The same procedures were conducted without oxidation with $KMnO_4$ in acidic medium for the binary mixtures. The mercury contents were calculated by using a calibration graph. The amount of Hg(I) ions were calculated by subtracting the amount of Hg(II) from total Hg [43,44]. The speciation results are extensively given in Table 4. The accuracy was verified by the Student's-test with calculated student's t-test value (1.59) less than the theoretical value (2.45, n: 8) at a confidence level of % 95. In addition, the statistical F-test was used for comparing the precision of the present method with those of the modified dithizone method. The $F_{4,4}$-test value at 95% confidence level did not exceed the theoretical value (4.28, n: 8) with a value of 1.92 for F-test, indicating no significant difference between the performance of the PAN method and the TAR method. It can

Table 3
Determination of inorganic mercury species in environmental water samples.

Sample	Added Hg(II) ($\mu g\,L^{-1}$)	The found value by calibration or standard addition approach[a]										The calculated Student's *t*- and *F*-values[c]
		PAN method					TAR method					
		Hg(II)			Total Hg[b]		Hg(II)			Total Hg[b]		
		$\mu g\,L^{-1}$	Recovery (%)	RSD (%)	$\mu g\,L^{-1}$	RSD (%)	$\mu g\,L^{-1}$	Recovery (%)	RSD (%)	$\mu g\,L^{-1}$	RSD (%)	
River water	–	6.73 ± 0.32	–	4.75	8.45 ± 0.34	4.26	6.78 ± 0.32	–	5.01	8.51 ± 0.38	4.46	0.169, 1.13 for Hg(II);
	50	56.76 ± 0.42	100.4	2.51	58.41 ± 0.45	2.32	57.02 ± 0.45	103.5	1.70	58.47 ± 0.42	2.03	0.181, 1.11 for total Hg
	100	106.58 ± 0.50	97.8	1.68	107.65 ± 0.63	1.64	106.93 ± 0.48	102.2	1.48	108.87 ± 0.52	1.62	
Lake water	–	4.13 ± 0.13	–	3.15	5.15 ± 0.12	2.33	4.17 ± 0.14	–	3.36	5.21 ± 0.15	2.49	0.33, 1.16 for Hg(II);
	50	53.93 ± 0.28	96.2	2.25	55.24 ± 0.21	2.30	53.98 ± 0.18	96.4	1.92	55.41 ± 0.25	2.35	0.54, 1.17 for total Hg
	100	104.27 ± 0.35	103.4	2.10	104.85 ± 0.29	1.79	104.11 ± 0.25	98.6	1.57	105.82 ± 0.45	1.68	
Tap water	–	3.67 ± 0.21	–	5.72	5.03 ± 0.18	4.18	3.62 ± 0.23	–	6.35	5.21 ± 0.15	4.84	0.254, 1.20 for Hg(II);
	50	53.55 ± 0.32	96.7	2.86	54.94 ± 0.24	3.46	53.57 ± 0.35	97.2	2.23	55.04 ± 0.21	2.36	1.396, 1.20 for total Hg
	100	103.78 ± 0.38	102.9	2.33	105.24 ± 0.32	2.84	103.76 ± 0.45	103.4	2.35	105.35 ± 0.34	2.28	
Dental waste water	–	12.24 ± 0.18	–	2.13	18.21 ± 0.19	1.85	12.32 ± 0.14	–	1.70	18.21 ± 0.19	1.72	0.45, 1.17 for Hg(II);
	50	62.12 ± 0.25	99.0	2.33	68.05 ± 0.24	2.20	62.51 ± 0.25	101.5	1.07	68.05 ± 0.24	1.08	0.36, 1.17 for total Hg
	100	112.45 ± 0.32	101.7	3.13	109.12 ± 0.34	2.12	112.52 ± 0.32	101.6	3.44	109.12 ± 0.34	2.32	
Chrome plating industry effluent	–	10.65 ± 0.27	–	2.31	16.21 ± 0.29	2.30	10.45 ± 0.27	–	1.95	16.12 ± 0.24	2.39	0.26, 1.41 for Hg(II);
	50	60.72 ± 0.25	100.6	2.13	66.05 ± 0.34	1.79	50.61 ± 0.25	101.5	1.57	65.94 ± 0.35	1.70	0.209, 1.13 for total Hg
	100	110.45 ± 0.32	98.2	2.54	116.12 ± 0.42	2.49	110.87 ± 0.32	104.0	3.02	116.72 ± 0.38	2.64	
Textile industry effluent	–	6.35 ± 0.17	–	1.77	10.42 ± 0.25	1.77	6.43 ± 0.20	–	2.05	10.61 ± 0.20	2.26	0.48, 1.08 for Hg(II);
	50	56.25 ± 0.24	98.4	1.76	60.27 ± 0.34	2.18	56.35 ± 0.31	98.8	1.56	60.54 ± 0.30	1.79	0.41, 1.20 for total Hg
	100	106.52 ± 0.32	102.6	2.68	110.63 ± 0.42	2.51	106.22 ± 0.36	96.7	3.11	110.43 ± 0.45	2.73	
[d]CRM, QC Metal LL3	–	6.43 ± 0.17	–	2.55	6.45 ± 0.21	2.43	6.42 ± 0.11	–	2.08	6.32 ± 0.16	2.11	0.09, 5.90 with PAN
	50	56.35 ± 0.20	98.4	2.25	56.55 ± 0.24	2.21	56.25 ± 0.22	97.4	1.69	55.25 ± 0.24	1.68	and 0.14, 4.92 with TAR
	100	106.50 ± 0.32	102.6	2.64	106.58 ± 0.29	3.26	106.12 ± 0.30	96.4	3.27	106.68 ± 0.39	3.58	for total Hg
[e]NIST, IAEA/W-4 (simulated fresh water)	–	2.43 ± 0.14	–	2.31	2.48 ± 0.13	2.71	2.38 ± 0.10	–	1.95	2.45 ± 0.14	2.56	0.19, 1.69 with PAN
	50	52.35 ± 0.22	96.7	2.13	52.54 ± 0.20	1.85	52.29 ± 0.20	96.2	1.57	52.56 ± 0.22	1.78	and 0.46, 1.96 with TAR
	100	102.50 ± 0.30	102.9	5.76	102.56 ± 0.34	5.24	102.50 ± 0.30	102.9	6.30	102.38 ± 0.36	5.71	for total Hg

[a] The average values and their standard deviations for five replicate measurements at 95% confidence level.
[b] The results indicate the total mercury values found by means of PAN and TAR methods after oxidation with $KMnO_4$ in acidic medium.
[c] The tabulated Student's *t*- and $F_{(4,4)}$ values are 2.78 and 6.39 for 95% confidence level and four degrees of freedom.
[d] The certified value is $6.48 \pm 0.51\ \mu g\,L^{-1}$ for total Hg.
[e] The certified value is $2.5 \pm 0.1\ \mu g\,L^{-1}$ for total Hg.

Table 4
The speciation results in binary mixtures containing Hg(I) and Hg(II) at known concentration ratios by two independent methods after oxidation under the optimized conditions.

Mixture (1)	After preconcentration of Hg(II) ions with PAN at 554 nm						
	Ratio	Added Hg(I) ($\mu g L^{-1}$)	[a]Total Hg(I) plus Hg(II) ($\mu g L^{-1}$)	[b]Found Hg(I) ($\mu g L^{-1}$)	RE (%)	RSD (%)	Recovery (%)
25 $\mu g L^{-1}$ Hg(II)	1:1	25.0	50.2 ± 2.5	25.2	+0.8	4.98	100.80
	1:5	125.0	150.8 ± 3.6	125.8	+0.64	2.39	100.64
	1:10	250.0	275.3 ± 3.7	250.3	+0.12	1.34	100.12
	1:15	375.0	400.2 ± 4.3	375.2	+0.05	1.07	100.05
Mixture (2)	After preconcentration of Hg(II) ions with TAR at 389 nm						
	Ratio	Added Hg(I) ($\mu g L^{-1}$)	Total Hg(I) plus Hg(II) ($\mu g L^{-1}$)	[a]Found Hg(I) ($\mu g L^{-1}$)	RE (%)	RSD%	Recovery (%)
125.0 $\mu g L^{-1}$ Hg(II)	1:1	125.0	250.98 ± 3.4	125.98	+0.78	1.35	100.80
	1:5	625.0	750.97 ± 5.2	625.97	+0.16	0.69	100.16
	1:10	1250.0	1375.53 ± 6.7	1250.53	+0.04	0.49	100.04
	1:15	1875.0	2000.40 ± 8.3	1875.40	+0.02	0.41	100.02

[a] The results indicate the total mercury values independently found by means of each method after oxidation with $KMnO_4$ in acidic medium. The average values and their standard deviation of three replicate surfactant sensitized spectrophotometric measurements at 95% confidence level.
[b] The results indicate the Hg(I) values found by subtracting Hg(II) concentration from total Hg concentration for five replicate measurements of each method.

Table 5
Comparison of the proposed CPE-spectrophotometric methods with the other analytical methods existed in literature.[a]

Method	[b]Chelating agent	Measurement wavelength, λ_{max}	Linear range ($\mu g L^{-1}$)	Detection limit ($\mu g L^{-1}$)	Preconcentration factor	RSD (%)	Media	Reference
CV-ICP-OES		–	–	0.45	–	–	–	[28]
CV-AAS		–	–	0.12	20	–	–	[45]
HPLC-CV-AFS	APDC	–	0.002–4.0	0.20	10	2.70	pH 3.5, Triton X-114	[46]
ET-AAS	5-Br-PADAP	–	0–10	0.10	–	4.00	pH: 8.5, Ponpe. 7.5	[47]
CV-AAS	DDTP	–	Up to 10 $\mu g L^{-1}$	0.12	–	4.60	0.1 M HCl medium, Triton X-114	[48]
On line FIA-spectrophotometry	Dithizone	500 nm	50–500	14.00	–	4.80	pH 1–3, Triton X-100	[37]
Spectrophotometry	Na-DDTC	–	4–240	0.53	–	1.90	pH 9.0, Triton X-100	[49]
Spectrophotometry	Rhodamine B hydrazide	556 nm	10–100	1.40	5	0.35	pH 5.0, Triton X-114	[50]
Spectrophotometry	Iodide	330 nm	10.0–400.0	3.00	19.5	2.51	H_2SO_4 medium, Triton X-114	[51]
Spectrophotometry	TMK	570 nm	5.0–80.0	0.83	–	0.27	pH 3.0 acetate buffer, Triton X-114	[52]
Spectrophotometry	PAN, TAR	554 nm and 389 nm	10.0–1000.0 and 50.0–2500.0	1.65 and 14.35	33.3 and 33.3	2.75 and 2.65	pH 9.0, pH 8.0 BR buf., Triton X-114	This study

[a] CV-ICP OES, cold vapor-inductively coupled plasma emission spectrometry. CV-AAS, cold vapor-atomic absorption spectrometry. HPLC-CV-AFS, high performance liquid chromatography-cold vapor atomic fluorescence spectrometry. ET-AAS, electrothermal atomic absorption spectrometry.
[b] TMK, Thio-Michler's Ketone. APDC, ammonium pyrolydine dithiocarbamate. Na-DDTC, sodium-diethyldithiocarbamate. PAN, 1-(2-pyridylazo)-2-naphthol. TAR, 4-(2-Thiazoylazo)resorcinol. 5-Br-PADAP, 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol. DDTP, o,o-diethyldithiophosphate.

be concluded that the comparison between the independent two methods will be accepted with a reasonable agreement (Table 3).

4. Conclusions

In the present study, two detection methods were developed for trace mercury ions. PAN and TAR was used as chelating ligand. The absorbances of the hydrophobic complexes were independently measured at 554 and 389 nm, respectively. These procedures allow determination of Hg species in the range of 10–1000 $\mu g L^{-1}$ for PAN and 50–2500 $\mu g L^{-1}$ for TAR. The detection limits of proposed methods 1.65 $\mu g L^{-1}$ for PAN and 14.5 $\mu g L^{-1}$ for TAR. The methods were successfully applied in the determination of mercury in environmental water samples and industrial effluents, and the results are statistically in good agreement with each other in terms of accuracy and precision. This spectrophotometric approaches based on CPE are simple and more sensitive than conventional spectrophotometric methods. These methods involve the use of an eco-friendly non-toxic surfactant rather than the organic solvents, which are conventionally used. The sensitivity of the methods has been compared with some of the reported micellar mediated CPE procedures (Table 5) [28,37,45–52]. These detection techniques such as CV-AAS, ET-AAS, CV-ICP-OES and HPLC-CV-AAS [28,45–48] are expensive, very complex, and are not available in every research laboratory in addition to requiring expertise in its field. The obtained preconcentration factor (33.3) is the best value according to the literature (especially in area of spectrophotometric detection). The detection limits of analytes are superior to those of preconcentration techniques [37,51] for analyses. Detection limit in the low $\mu g L^{-1}$ range with especially the PAN method is sufficient for pollution control measurements in ambient waters, as we have demonstrated by testing certified reference materials and real wastewater from industrial origin with good recoveries. Potential applicability to environmentally water samples such as hospital effluent water, lake water, river water as well as tap water has been

demonstrated with the accurate results obtained for fortified samples analysis. The methods have the following advantages: simple, rapid, reproducible, sufficient sensitivity, wide linear range, and low analysis cost. These methods can be applied to trace mercury determination in every analysis laboratory having a conventional spectrophotometry without needing any expensive instrument.

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