

## Cloud-point extraction/preconcentration on-line flow injection method for mercury determination

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### Abstract

A cloud-point extraction/preconcentration (CPE/P) step is incorporated on-line into a flow injection system which is used to determine low levels of Hg(II) added to natural water samples. The analyte is complexed with dithizone. A solid reagent column (SRC) is used to prepare the reagent on-line by using 5% (v/v) Triton X-100 solution as solvent. The CPE/P is carried out by using the non-ionic surfactant Triton X-100. After obtaining the cloud-point on-line, the surfactant-rich phase containing the complex is collected in a mini column packed with cotton wool. Then, a hot water stream is passed through the column to elute the complex and the absorbance is measured at 500 nm.

All the flow and chemical variables are optimized and the enhancement factor for the system is estimated. The calibration is linear over the range 0.05–0.5  $\mu\text{g ml}^{-1}$ , the R.S.D. is 4.8%, the limit of detection (signal:noise = 3) is 0.014  $\mu\text{g ml}^{-1}$  and the sample throughput is 30  $\text{h}^{-1}$ . An open/closed system is used to eliminate the interference of iron(III).

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### 1. Introduction

Mercury(II) is considered a chemical pollutant and although it is found at low concentration, its toxicity can be dangerous. So, the determination and monitoring of this analyte is becoming increasingly interesting in environmental samples.

Several methods for ultra-trace mercury analysis in environmental samples are used including atomic absorption spectrometry with cold vapor [1–3], inductively coupled plasma mass spectrometry [4]. Mercury determination in natural water samples is of a great interest for pollution control. However, mercury is at such a low concentration in this kind of sample that the determination requires a preconcentration step of the analyte before applying the above mentioned techniques.

To carry out the separation and preconcentration of the analyte, a cloud-point extraction was used in this study. This method is becoming an important and practical application of the use of surfactants in analytical chemistry

[5]. Such an extraction offers a convenient alternative to more conventional extraction systems. Aqueous solutions of non-ionic and zwitterionic surfactants possess the ability to decrease their solubility quickly and become turbid when heated above a temperature referred to as the cloud-point [6,7]. For higher temperatures, two distinct phases are formed, one consisting almost totally of the surfactant and the other containing a small portion equal to the critical micellar concentration (cmc) [8,9]. The mechanism by which this separation occurs is attributed to the rapid increase in the aggregation number of the surfactant's micelles, as a result of the increase of temperature or to critical phenomena [10,11]. During their formation, it was proved that the surfactant's micelles entrap several hydrophobic substances, isolating them from the bulk aqueous solution [8,12].

Cloud-point extraction (CPE/P) has been shown to be an effective sample pretreatment approach for improving sensitivity and selectivity prior to flow injection analysis (FIA) as well as to liquid chromatography (LC). Fernández Laespada et al. [13] were the first to recognize the advantages of combining CPE/P with FIA but the cloud-point methodology was performed off-line in their experiments. The ability to perform CPE/P on-line in a FIA system has been reported by Fang et al. [14] for the first time. The authors evaluated

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the analytical performance of the on-line CPE–FIA system by using hematoporphyrin as a model test compound.

In the present paper, an automated method for spectrophotometric determination of Hg(II) in water samples is proposed. With the FIA system developed it was possible: (a) to carry out the pretreatment of the sample on-line to eliminate Fe(III) interference, (b) to prepare the reagent (dithizone) on-line by using a solid reagent column (SRC) [15], (c) to preconcentrate the analyte on-line by using a continuous CPE/P and (d) to obtain a spectrophotometric signal of the analyte for its quantification.

The use of Triton X-100 as a solvent for the preparation of dithizone reagent was an important advantage because this surfactant was also used for cloud-point formation.

## 2. Experimental

### 2.1. Instrumentation

Spectrophotometric measurements were performed on a Hewlett Packard 8452A diode array spectrophotometer equipped with a Hellma 178712-QS flow cell with an inner volume of 8  $\mu$ l and 10 mm light path. Ismatec and Gilson Minipuls 3 peristaltic pumps were used. A Rheodyne 5041 injection valve was employed. All the reaction coils were of PTFE tubing (i.d. 0.5 mm). The mini-column consisted of a piece of glass tubing (2.5 cm  $\times$  4 mm i.d.) filled with cotton wool. A JULABO thermostatic bath was used to reach the cloud-point temperature.

### 2.2. Reagents and solutions

Analytical grade chemical reagents were always used as well as ultra pure water. The Hg(II) stock solution was prepared by dissolving 0.1484 g of HgCl<sub>2</sub> (Merck)

in 1000 ml of water. Standard solutions were prepared by appropriate dilution of the stock solutions. A 5% (v/v) Triton X-100 solution was prepared in 0.1 M HCl. Dithizone (Riedel-de-Haën): a SRC was prepared by filling a dark Tygon<sup>®</sup> tube with the compound. Na<sub>2</sub>SO<sub>4</sub> solution (1.25 M) was prepared by dissolving 44.375 g in 250 ml of deionized water.

### 2.3. Procedure

The FIA system for Hg(II) determination is shown in Fig. 1. Dithizone solution was generated on line by using the SRC, when a stream of Triton X-100 5% (v/v) in 0.1 M HCl was passed through the column.

This reagent merged with 0.3 M HCl into reactor R<sub>1</sub> to obtain a suitable pH for complex formation with Hg(II) in the sample.

The Hg(II)–dithizone complex was formed in reactor R<sub>2</sub>. The complex solution was merged with the Na<sub>2</sub>SO<sub>4</sub> solution in R<sub>3</sub>. The Na<sub>2</sub>SO<sub>4</sub> solution was used to decrease the cloud-point temperature. So, reactor R<sub>3</sub> was kept in a thermostat at 60  $\pm$  0.2  $^{\circ}$ C; this was the optimum temperature to obtain the cloud-point.

A glass mini-column packed with cotton wool (CC) replaced loop of the injection valve, to collect the surfactant-rich phase containing the complex. The aqueous phase went through the CC column to waste. After 1 min, the valve was switched and the eluent stream (water at 60  $^{\circ}$ C) went through the CC column, and the complex was carried towards the flow cell.

A single bead string reactor (SBSR) was placed between the injection valve and the flow cell in order to promote mixing between the surfactant-rich phase and the eluent. Finally, this stream was passed through a knotted reactor R<sub>4</sub> and the spectrophotometric signal was measured at 500 nm.

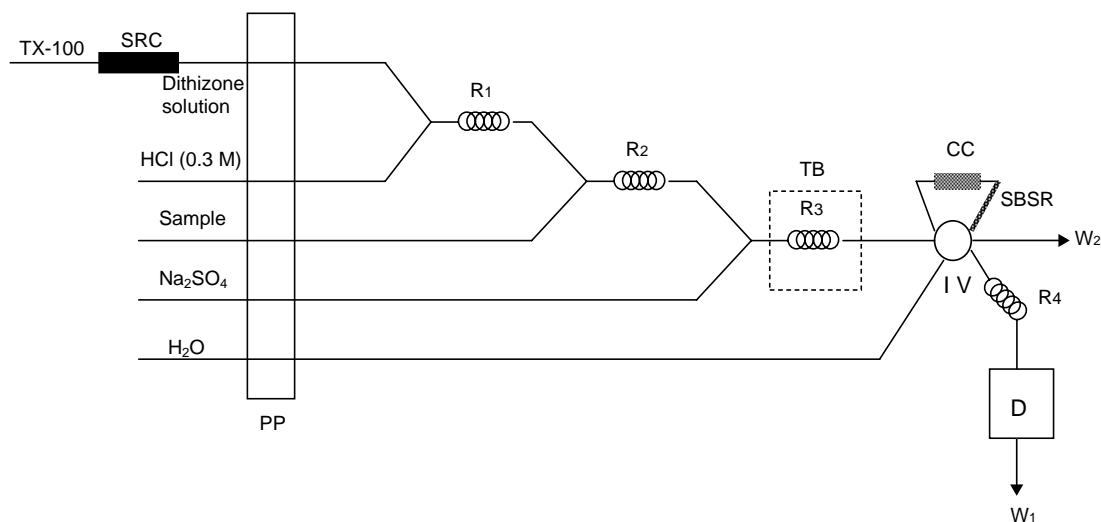


Fig. 1. CPE/FIA manifold for Hg(II) pre-concentration and determination. PP: peristaltic pump, SRC: solid reactor column, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub>: reactors, SBSR: single bead string reactor, IV: injection valve, W<sub>1</sub> and W<sub>2</sub>: waste, CC: collection column, TB: thermostatic bath, D: detector.

### 3. Results and discussion

The temperature used to carry out the CPE/P of the dithizone–Hg(II) complex by using Triton X-100 solution was  $72 \pm 0.2^\circ\text{C}$ . This temperature produced bubbles in the FIA system, so the signals were not reproducible. To avoid this effect a salting-out agent was added in order to decrease the temperature of the cloud-point [16]. Different salting-out agents (NaCl,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{Na}_2\text{SO}_4$ ) were tested at  $60^\circ\text{C}$ . The dithizone–Hg(II) complex became unstable when NaCl and  $(\text{NH}_4)_2\text{SO}_4$  were used. Therefore,  $\text{Na}_2\text{SO}_4$  was selected. Different concentrations of the selected agent were tested between 1 and 2 M. The optimum value was 1.25 M.

#### 3.1. Optimization of FIA and chemical variables

The variables influencing the performance of the method were studied and optimized in order to obtain a high signal and good reproducibility, by using the univariate method. The studied range of the FIA variables and their optimum values are listed in Table 1.

Dithizone is soluble in organic solvents and, also, in Triton X-100 solution in acidic medium [17]. Moreover, the reagent prepared with the surfactant is unstable to light exposure. The SRC developed in our laboratory is very convenient to prepare the reagent solution on-line.

The SRC was prepared with a dark Tygon<sup>®</sup> tube (length: 5 cm; i.d.: 3 mm) packed with solid dithizone. Different percentages of Triton X-100 were tested (1–10%) in order to establish on line a suitable concentration of this reagent. The optimum value was 5%. The Triton X-100 was always prepared in HCl (0.1 M).

The concentration of the HCl stream was important for this method in order to obtain a suitable pH 1–3 for complex formation. The concentration range of HCl tested was (0.1–0.5 M) and the optimum value was 0.3 M.

Table 1  
Optimization of FIA variables

Variable	Studied range	Optimum value
Triton X-100 flow rate ( $\text{ml min}^{-1}$ )	0.40–0.76	0.54
Dithizone flow rate ( $\text{ml min}^{-1}$ )	0.40–0.76	0.54
HCl flow rate ( $\text{ml min}^{-1}$ )	0.40–1.10	0.76
Sample flow rate ( $\text{ml min}^{-1}$ )	0.40–1.50	1.10
$\text{Na}_2\text{SO}_4$ flow rate ( $\text{ml min}^{-1}$ )	0.40–1.50	1.10
Eluent flow rate ( $\text{ml min}^{-1}$ )	1.00–3.80	1.00
Reactor length $R_1$ (mm)	300–900	600
Reactor length $R_2$ (mm)	100–400	300
Reactor length $R_3$ (mm)	600–2900	1900
Knotted reactor length $R_4$ (mm)	270–600	340
SBSR length (cm)	4.5–7.5	6.5
SBSR inner diameter (mm)	0.8–1.4	1.1
CC length (cm)	1.5–3.5	2.5
CC inner diameter (mm)	2–5	4
Cotton wool dry weight (mg)	70–110	90
Loading time (s)	30–80	60

#### 3.2. Optimization of the separation/preconcentration process

The homemade mini-column was packed with different filtering materials in order to obtain phase separation by entrapping larger surfactant aggregates and allowing smaller components within the aqueous medium to pass through.

When glass wool was used, the retention of the surfactant-rich phase was not efficient, and the surfactant aggregates passed through the column. Cigarette filter was also tested and the adsorbed dithizone could not be eluted. Finally, cotton wool was found to provide a satisfactory surfactant-rich phase retention and the best reproducibility.

The amount of cotton wool in the mini-column is important for collecting the surfactant-rich phase. So, different amount of dry cotton wool was tested (70, 90 and 110 mg). The best results were obtained when ca. 90 mg was used. A greater amount of cotton wool produced pressure in the system and, when a smaller amount was used, collection was not efficient. The length and inner diameter of a glass mini-column were also optimized. With 2.5 cm effective length and 4 mm inner diameter, the best peak shape and reproducibility were obtained (Table 1).

In order to elute the surfactant-rich phase, deionized water at different temperatures was tested ( $25$ – $80^\circ\text{C}$ ). The best reproducibility was obtained by using a stream of water at  $60^\circ\text{C}$ .

The enhancement factor (EF) for this system was estimated by using the equation  $\text{EF} = 2A_e/A_s$  [18]; where  $A_e$  and  $A_s$  are the absorbance of the collected solution with and without preconcentration, respectively. The obtained EF for  $0.3 \text{ mg l}^{-1} \text{ Hg}^{2+}$  was 6.

#### 3.3. Interferences

The effect of foreign ions on the determination of  $0.3 \mu\text{g ml}^{-1} \text{ Hg}^{2+}$  was studied. The common ions at the usual

Table 2  
Tolerance limits of interfering ions

Tested ion	Tolerance limit ( $\text{mg l}^{-1}$ )
$\text{Cd}^{2+}$	2
$\text{Pb}^{2+}$	2
$\text{Zn}^{2+}$	2
$\text{Ni}^{2+}$	2
$\text{Ca}^{2+}$	>350
$\text{Mg}^{2+}$	>230
$\text{K}^+$	>900
$\text{NH}_4^+$	>100
$\text{Cu}^{2+}$	<1
$\text{Fe}^{3+}$	<1
$\text{Al}^{3+}$	<1
$\text{CO}_3^{2-}$	>500
$\text{Br}^-$	>500
$\text{Cl}^-$	>900
$\text{F}^-$	5
$\text{NO}_3^-$	>900

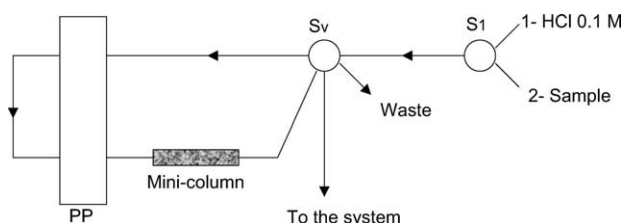


Fig. 2. Open-closed FIA manifold for sample pre-treatment. PP: peristaltic pump, Sv: selection valve, V1: six-position rotary valve.

concentrations found in natural water samples, as well as those ions that form complexes with dithizone, were tested. The tolerance limits are shown in Table 2.  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  caused interference.

To remove up to  $0.3 \text{ mg l}^{-1}$  aluminum, sodium tartrate ( $6.6 \times 10^{-3} \text{ M}$ ) was used as a masking agent. The interference of copper ( $0.3 \text{ mg l}^{-1}$ ) was eliminated by adding sodium thiosulphate ( $1.12 \times 10^{-2} \text{ M}$ ) to the water samples.

The interference of iron could not be eliminated by using masking agents, so an additional open/closed FIA system was developed for the pre-treatment of the sample (Fig. 2). A mini-column was packed with a Dowex 50W X-16 resin to remove the iron from the sample. The resin was regenerated by passing  $0.1 \text{ M HCl}$  through the mini-column for 5 min. The sample was pumped into the system and, when the manifold was loaded, the selecting valve (Sv) was switched to close the manifold. The sample plug was trapped to recycle through the closed system for 7 min. The  $\text{Fe(III)}$  was retained in the resin during this time. Then the Sv valve was switched again to open the manifold and the sample stream, free of iron interference, was pumped to the system. The resin must be regenerated before loading the next sample. The open/closed FIA system was optimized. The optimum values for the mini-column size,

Table 3  
Optimization of FIA variables of open/closed system

Variable	Studied range	Optimum value
Mini-column length (cm)	4.0–7.0	6.0
Inner diameter (mm)	3–4	4
Inner volume of the closed circuit (ml)	4–7	5
Flow rate in the closed circuit ( $\text{ml min}^{-1}$ )	1.50–2.40	2.10
Time to recycle the sample in the closed circuit (min) (for samples containing $0.3 \text{ mg l}^{-1} \text{ Fe(III)}$ )	1–4	2.5
Time to recycle the sample in the closed circuit (min) (for samples containing $0.5 \text{ mg l}^{-1} \text{ Fe(III)}$ )	4–8	7
Time to regenerate the resin (min)	3–7	5

the time to regenerate the resin and the flow rate for the pre-treatment system are shown in Table 3. In this way, the effect of  $0.5 \text{ mg l}^{-1} \text{ Fe(III)}$  was eliminated.

The efficiency of the open/closed system was proved with spiked samples. Two  $\text{Hg}^{2+}$  standard solutions ( $0.3 \text{ } \mu\text{g ml}^{-1}$ ) were spiked with  $0.3$  and  $5 \text{ mg l}^{-1}$  of  $\text{Fe(III)}$ . These samples were analyzed by the proposed method and the recoveries were close to 100% ( $0.303$  and  $0.307 \text{ } \mu\text{g ml}^{-1} \text{ Hg}^{2+}$ , respectively).

### 3.4. Analytical performance

The calibration graph was linear over the range  $0.05$ – $0.5 \text{ } \mu\text{g ml}^{-1} \text{ Hg}^{2+}$  ( $A = (0.170 \pm 0.003) [\text{Hg}^{2+}, \text{ } \mu\text{g ml}^{-1}] + (0.003 \pm 0.0009)$ ;  $R^2 = 0.9997$ ,  $n = 6$ ).

The reproducibility of the proposed method was estimated by running seven calibration graphs on different days and with different conditions (standard solution, reagent solution, etc.). The mean of the slope was  $0.170$  and the R.S.D. was  $4.8\%$ .

Table 4  
Determination of mercury(II) added to real samples

Sample	Hg(II) added ( $\mu\text{g ml}^{-1}$ )	Proposed method		Reference method	
		Hg(II) found ( $\mu\text{g ml}^{-1}$ )	Recovery (%)	Hg(II) found ( $\mu\text{g ml}^{-1}$ )	Recovery (%)
1 to 6	None	None		None	
1	0.150	$0.157 \pm 0.004^a$	$104.9 \pm 2.3$	$0.152 \pm 0.006$	$101.3 \pm 3.2$
1	0.350	$0.357 \pm 0.003$	$102.0 \pm 0.9$	$0.355 \pm 0.005$	$101.4 \pm 1.8$
2	0.150	$0.147 \pm 0.004$	$98.0 \pm 2.7$	$0.145 \pm 0.008$	$96.7 \pm 2.2$
2	0.350	$0.360 \pm 0.005$	$102.9 \pm 1.5$	$0.362 \pm 0.004$	$103.4 \pm 0.6$
3	0.150	$0.156 \pm 0.006$	$104.0 \pm 4.0$	$0.152 \pm 0.009$	$101.3 \pm 1.5$
3	0.350	$0.362 \pm 0.006$	$103.4 \pm 1.7$	$0.337 \pm 0.005$	$96.3 \pm 1.2$
4	0.150	$0.151 \pm 0.005$	$100.9 \pm 3.4$	$0.154 \pm 0.004$	$102.7 \pm 3.3$
4	0.350	$0.347 \pm 0.001$	$99.1 \pm 0.3$	$0.340 \pm 0.005$	$97.1 \pm 0.8$
5	0.150	$0.147 \pm 0.006$	$97.8 \pm 4.3$	$0.147 \pm 0.007$	$98.0 \pm 4.0$
5	0.350	$0.353 \pm 0.006$	$101.0 \pm 1.9$	$0.352 \pm 0.006$	$100.6 \pm 1.7$
6	0.150	$0.157 \pm 0.000_6$	$104.5 \pm 0.4$	$0.150 \pm 0.010$	$100.0 \pm 1.8$
6	0.350	$0.347 \pm 0.001$	$99.1 \pm 0.3$	$0.325 \pm 0.005$	$92.9 \pm 2.5$

(1) Sample of Colorado River (Paso Alsina, Río Negro Satate, Argentine); (2) sample of Paso Piedras Dike (Bahía Blanca, Bs. As. State, Argentine); (3) drinking water of Bahía Blanca (Bs. As. State, Argentine); (4) sample of Negro River (C de Patagones, Río Negro State, Argentine); (5) sample of well water from San Cayetano (Bs. As. State, Argentine); (6) sample of Paso Piedras Dike (Bahía Blanca, Bs. As. State, Argentine).

<sup>a</sup> Standard deviation for  $n = 3$ .

The repeatability of the method was calculated as the R.S.D. of the maximum peak heights, for 12 replicates ( $0.3 \mu\text{g ml}^{-1} \text{Hg}^{2+}$ ) injected in duplicate. The result obtained was 0.52%. The sample throughput was  $30 \text{ h}^{-1}$ .

When the open/closed FIA manifold was used, the same calibration graph was obtained. The repeatability and sample throughput were 2.3% and  $4 \text{ h}^{-1}$  to eliminate up to  $0.5 \text{ mg l}^{-1} \text{Fe(III)}$ . The detection limit ( $S/N = 3$ ) was  $0.014 \mu\text{g ml}^{-1}$ .

### 3.5. Analysis of real samples

The proposed method for the determination of mercury was applied to some water samples. These real samples showed that mercury was not detectable in them, so they were spiked with a standard mercury solution. In order to assess the quality of the results obtained, they were compared with those provided by inductively coupled plasma-atomic emission spectrometry.

Sodium thiosulphate solution and sodium tartrate solution were added to the samples in order to eliminate possible Cu(II) and Al(III) interferences, respectively. Samples were also passed through the open/closed FIA manifold to eliminate possible Fe(III) interference.

The recovery values obtained from mercury added to natural water samples are shown in Table 4. A *t*-test to evaluate the percentage recovery was used. The results revealed no significant differences between  $\bar{R}$  (%) and 100%. Then, taking in account that both variances are equal ( $F_{\text{calculated}} = 1.54$  and  $F_{\text{critic}}(95\%, 11) = 3.68$ ), a comparison between  $\bar{R}$  (%) of the proposed method and  $\bar{R}$  (%) of the reference method was made. The results showed no significant statistical differences ( $t_{\text{calculated}} = 1.85$  and  $t_{\text{critic}}(95\%, 22) = 2.07$ ).

## 4. Conclusions

CPE/P is an easy, safe and inexpensive methodology for separation and preconcentration of trace metals in aqueous solutions. The on-line incorporation of CPE/P into a FIA system represents an important advantage, because it avoids all manual operations.

Triton X-100 surfactant used in the proposed method had a double purpose, as a solvent for dithizone in the SRC and,

for the extraction and preconcentration by the cloud-point technique.

The developed CPE/P-FIA system with spectrophotometric detection for determination of mercury provides good quality results in terms of accuracy and precision and they show good agreement with those obtained by using the reference method. The CPE/P preconcentration step is a rapid and effective methodology, so the method has a suitable sample throughput and presents a detection limit about  $14 \text{ ng ml}^{-1}$ .

The proposed method to determine mercury is simple, fast and inexpensive. With this automated method, it is possible to do on-line pretreatment of the sample (separation and preconcentration of the analyte) and an inexpensive detector as spectrophotometer is used.

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