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Exploiting the bead injection concept for sequential determination of copper and mercury ions in river-water samples

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Abstract

A procedure involving bead-injection concept and sequential determination of copper and mercury ions in river-water samples is proposed. The method is based on the solid-phase extraction of both metal ions on the same beads surface (Chelex 100 resin) and in their subsequent reaction with the colorimetric reagents (APDC and Dithizone for copper and mercury ions, respectively). For this task, a resin mini-column is established in the optical path by the selection, introduction and trapping of a defined volume of the Chelex-100 resin beads suspension in the flow system. The passage of the sample solution through the resin mini-column promotes the sorption of Cu(II) ions and, making the APDC colorimetric reagent flows through the beads, the formation of the coloured complex on the solid phase surface occurs. The absorbance of the formed APDC–Cu complex is then monitored at 436 nm and the spent beads are discarded. Packing another resin mini-column in the flow cell and repeating the concentration step it is possible to carried out the mercury determination by using Dithizone as reagent. The absorbance of the Dithizone–Hg complex is monitored at 500 nm. After each measurement, the spent beads are wasted and a new portion of fresh one is trapped in the system, letting it ready for the next measurement. The bead injection system is versatile and can be used to concentrate different sample volumes, which permits the determination of a wide range of copper and mercury ions concentrations. When the sample-selected volumes are 100 and 1000 μl the analytical ranges were 5.0 up to 500.0 $\mu g\,l^{-1}$ and 2.5 up to 30.0 $\mu g\,l^{-1}$ for Cu(II) and Hg(II) ions, respectively. Under these conditions, the detection limit was estimated as 0.63 and 0.25 $\mu g\,l^{-1}$ for copper and mercury ions determination. The system consumes 2 mg of Chelex 100 resin beads, 0.20 mg of APDC or 1.25 mg of Dithizone per determination and the traditional organic solvent extraction methodology, normally used in connection with APDC and Dithizone reagents, is not used here which permits to classify the present method as green.

Keywords: Bead injection; River-water; Dithizone

1. Introduction

Simultaneous or sequential determination always was a dream in analytical chemistry. A technique that could analyzes a sample without any step devoted to sample preparation and, that at the same time, could analyze all the species present in the sample can be considered as an ideal analytical technique. Unfortunately, this technique was not discovered yet. We have some analytical techniques that are close to this dream, as the neutronic activation analysis [1] and the atomic X-ray spectrometry [2]. These techniques can be applied to direct solid sample analysis but, working with the first one, sometimes it is necessary to have a font with a high flux of neutron, which means, a nuclear reactor. It should be considered that depend on the analyte to be determined the counting step its takes some days. The X-ray spectrometry is fine to determination of chemical species in solid samples but, for liquid ones, a prior sample treatment is required furthermore, the excitation of some elements is not easy attained. It should be stressed that any cost analysis is taken account here, and for a lot of routines laboratories the cost of the neutronic activation analysis and X-ray spectrometry can be considered impeditive.

As the ideal analytical technique does not exist, the best that we can do is develops simultaneous or sequentially analytical methods with the minimal sample manipulation or, if sample manipulation was necessary, we should work with a solution management machine, that nowadays, it means

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exploitation of flow analysis [3]. This tendency can be verified since the advent of the segmented flow analyzers and in the subsequent proposal of the sequential multiple analyzers [4,5] that was conceived aiding to carried out simultaneous determinations. The same tendency is observed in modern flow based procedures as flow injection and sequential injection analysis [6–9].

The association of sequential injection analysis [10] and solid phase spectrophotometry [11] leads to the bead injection concept [12], which is based on the introduction and trapping of functionalised beads in the flow conduits. The presence of the solid phase in the sequential injection system contributes to give some new characteristics to it, among them, two properties should be emphasised: first, the solid phase acts as solid phase extractor, as the analyte is absorbed on the beads surface and is separated from the sample matrix increasing the sensitivity and selectivity [13]; second, the solid-phase acts as solid reagent, as the absorbed species react with the bead surface promoting a physical change in it that can be monitored by using an adequate detector. For this last task, sometimes it is necessary the addition of an auxiliary reagent [14,15]. The beads surface can be renewed after each cycle, avoiding lost in the retention efficiency due to elution of the functional group or poisoning the surface by the sample components.

Several analytical methodologies exploiting the bead injection concept have been proposed [16–18] demonstrating that the new concept is feasible. Based on this concept and in the actual tendency where the development of simultaneous and/or sequential analytical methods is a reality, the present paper describes the development of bead injection system for sequential determinations of mercury and copper ions based on solid phase extraction which eliminates the usage of organic solvent extraction.

The traditional methods for copper and mercury determinations [19,20] is based on the reaction of the metal ions with a chelanting reagent, and in the subsequent extraction of the hydrophobic formed chelates with an organic solvent. As the volume of the organic solvent can be smaller than the aqueous sample solution, the metallic ions are concentrated in the organic phase, where they are monitored. In this way, the limit of detection is better and the possible interferant substances present in the original sample can be discarded in the aqueous phase. Initially, spectrophotometric detectors were used in organic solvent extraction methodologies [19,20]; nowadays the usage of more modern analytical instruments as atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry is a reality [21,22], which improve the detection limit but increasing the cost of the equipments used in the analysis.

Recently, efforts have been made to avoid the use of organic solvent in chemical procedures; in analytical chemistry when the sample to be analysed presents analyte concentrations lower than the limit of detection the new elected pre-concentration step normally is the solid phase extraction [23]. For the task, a chelanting agent is immobilized in a solid support and the metallic ions present in the samples are absorbed on its surface. After the elution step, which is made with a small volume of an adequate reagent, modern analytical techniques are used to detect the analyte [23,24]. Normally the active reagent immobilized on the solid phase are the same chelanting reagents that were used before in the liquid–liquid extraction, thus the selectivity and sensitivity are improved in the same way that in classical liquid–liquid extraction but here the hazardous organic solvents are not used.

Following this tendency, in the present work solid phase extraction is exploited for copper and mercury determination in river water samples, but the analytes that are retained in the solid phase are not eluted, they are directly spectrophotometric monitored on the solid surface. With this strategy, the selectivity is comparable to the solid phase extraction procedure and the sensitivity is better as there is not the presence of the eluant that dilutes the analytes. The usage of organic solvent extraction is avoided, thus the method can be considered as green although of the use of Dithizone and pyrrolydine dithiocarbamete (APDC) as reagents.

2. Experimental

2.1. Reagent and solutions

The Dithizone reagent solution (0.005% (w/v) Dithizone, pH 10.7) was daily prepared by dissolving 5 mg of Dithizone up to 100 ml with a 2.5% (w/v) NH_3.

The APDC reagent solution was 0.1% (w/v) was prepared dissolving 0.10 g of ammonium pyrrolidino dithiocarbamate up to 100 ml with water.

The mercury standard stock solution, 1000 mg l^{-1}, was prepared by dissolving 1.618 g $Hg(NO_3)_2$ up to 1000 ml with 0.014 mol l^{-1} HNO_3, and the working standard solutions (2.5–25.0 μg l^{-1} Hg in 0.014 mol l^{-1} HNO_3) were daily prepared by proper dilution of the stock solution.

The copper standard stock solution, 1000 mg l^{-1}, was prepared by dissolving 0.3930 g of $CuSO_4{\cdot}5H_2O$ in 15 ml of nitric acid, 1.0 mol l^{-1}, and making up the volume to 100 ml with water. The working standard solutions (5.0–500.0 μg l^{-1} Cu in 0.014 mol l^{-1} HNO_3) were daily prepared by proper dilution of the stock solution.

A chelanting resin suspension was prepared by adding 10 ml of deionised water to 0.25 g of Chelex 100 resin (sodium form, 200–400 mesh) purchased from Bio-Rad. The suspension (Chs) was kept under mechanical agitation.

2.2. Samples

River-water samples were collected, stored in polyethylene bottles and preserved by the addition of ca. 1 ml of concentrated HNO_3 per liter of the water sample.

Before analysis, 100 ml of each sample was transferred to digestion tubes and evaporated almost to dryness. Then, 1 ml

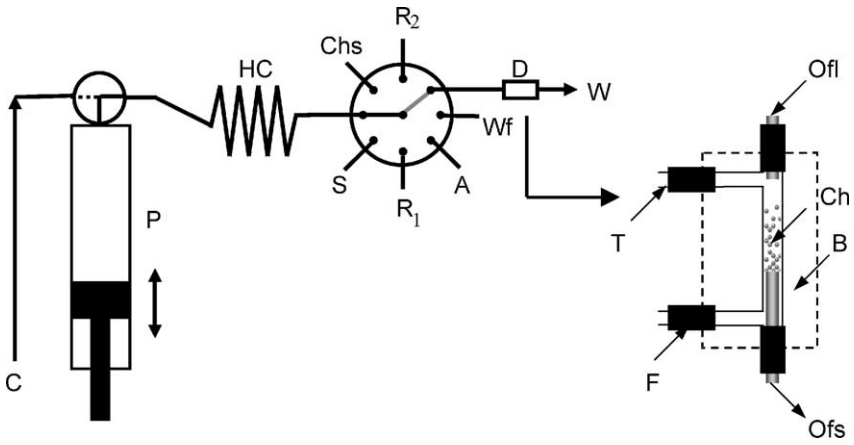

Fig. 1. Sequential injection system. C: carrier stream, Chs: Chelex beads suspension, S: sample, R_1: Dhitizone reagent, A: acidic solution, HC: holding coil, P: syringe pump, D: detector at 500 nm, Wf: waste flask and W: waste. Detail: Perspex flow cell, Ofs and Ofl: optic fiber to spectrophotometer and optic fiber from light source, Ch: Chelex 100 resin beads, T: PTFE tubes, F: fittings and B: Perspex block.

of concentrated HNO_3 was added to the tubes and evaporated almost to dryness; the samples recovered in 0.014 mol l^{-1} HNO_3.

2.3. Apparatus

The sequential injection system was a FIAlab-3000 (Alitea, USA, Medina, WA), equipped with a UV–vis CCD spectrophotometer and a LS1 tungsten halogen light source (Ocean Optics Inc., Dunedin, FL) coupled to a tubular flow cell (optical path 10 mm, inner volume 20 μl) by a fiber optic cable of 400 μm. Reactors and transmission lines were built up with 0.8 mm i.d. PTFE tubes.

2.4. Procedure

The operation of the sequential injection system (Fig. 1) is shown in Table 1 and Fig. 2. Initially, 40 μl of a resin suspension were aspirated to the holding coil, and then directed towards the flow cell, where the beads were packed inside the optical path. Then, a defined sample volume was selected and directed to the chelanting resin mini-column, where the mercury and copper ions are retained. An acidic solution (50 μl) was then aspirated to the holding coil and making it to flows through the resin mini-column partial elution of copper ions and the elution of some potential interferant ions occurs. The subsequently selection of 50 μl of the

Table 1
System operation

Step	Event	Port	Time(s)	Flow direction	Flow rate (μl/s)	Volume (μl)
1	Beads aspiration	Chs	1.4	Reverse	100	40
2	Propels beads towards detector (packing the mini-column)	D	2.0	Forward	100	200
3	Sample aspiration	S	10	Reverse	100	1000
4	Propels sample through the resin mini-column	W	60	Forward	20	1200
5	Sampling acidic solution	D	1.0	Reverse	50	50
6	Propels acidic solution through the resin mini-column	D	2.5	Forward	20	50
7	Sampling Dithizone reagent	R_1	2.5	Reverse	20	50
8	Pumping Dithizone reagent through resin mini-column and measurement of the analytical signal	D	50	Forward	10	500
9	Aspirating spent beads to the holding coil	D	3.0	Reverse	100	300
10	Direct spent beads to the recovery flask	Wf	2.0	Forward	200	400
11	Beads aspiration	Chs	1.4	Reverse	100	40
12	Propels beads towards detector (packing the mini-column)	D	2.0	Forward	100	200
13	Sample aspiration	S	2.0	Reverse	50	100
14	Propels sample through the resin mini-column	D	12	Forward	10	120
15	Sampling the APDC reagent	R_2	2.0	Reverse	100	200
16	Pumping the APDC through the resin mini-column and signal measurement	D	6.0	Forward	40	240
17	Aspirating spent beads to the holding coil	D	3.0	Reverse	100	300
18	Direct spent beads to the recovery flask	Wf	2.0	Forward	200	400

The steps to Hg(II) and Cu(II) sequential determination are listed here. The same steps can be visualized in Fig. 2.

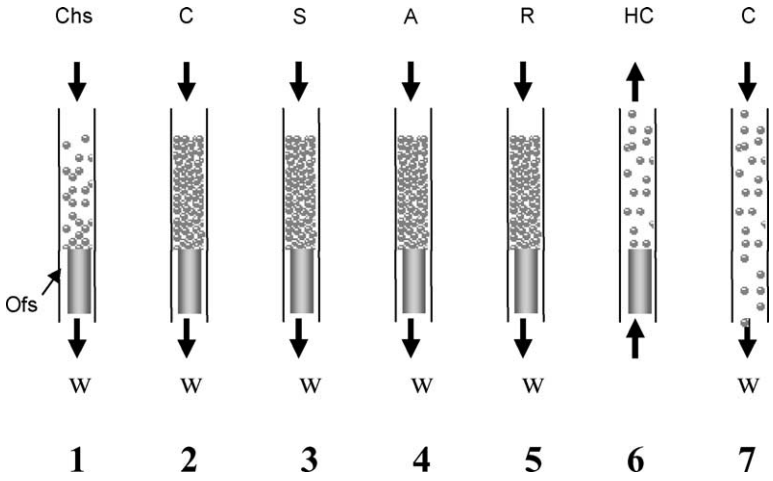

Fig. 2. Sequence of events for sequential determination of Hg(II) and Cu(II). (1) Packing the beads, (2) the in-line packed resin mini-column, (3) the packed beads are perfused by the sample, (4) the packed beads are perfused by the acidic solution (only for mercury determination), (5) reagent (Dithizone or APDC) is propelled through the packed beads and the detector, (6) the spent beads are aspirated to holding coil, (7) the spent beads are directed to the waste flask. Ofs: optical fiber. Other symbols see Fig. 1.

Dithizone reagent and, making it to flows through the flow cell leads to the formation of the complex Hg–Dithizone on the bead surfaces, which is monitored at 500 nm. The spent beads are then aspirated back to the holding coil and directed to the waste flask, located in a port of the selection rotary valve. At this point, the mercury ions were determined. Packing again a new resin mini-column in the optical path, let the system ready to carry out the copper ions determination. In the sequence, a defined sample volume was selected and it is forced to flows through the resin mini-column, where the copper and mercury ions are retained. The selection of 200 μl of the APDC reagent and, directing it towards the flow cell, results in the formation of a colored complex Cu–APDC on the bead surfaces, which is monitored at 436 nm. Here, the spent beads are discarded again and the system is ready to process a new sample.

2.5. System optimization

The main relevant parameters, such as the measurement wavelengths, sample and reagent flow rates, chelanting resin quantity, composition of the reagents, reagent and sample volumes were investigated.

For mercury ions determination, the wavelength for absorbance measurements was selected by evaluating the Dithizone and complex Hg–Dithizone spectra, which were obtained by processing the blank and the 50.0 $\mu g\,l^{-1}$ Hg(II) standard solution. For copper ions, the wavelength for absorbance measurements was fixed at 436 nm as described elsewhere [13].

Finally, the main analytical characteristics, such as the sampling frequency, reagent consumption, linear analytical range, precision and accuracy were evaluated by processing river-water samples in the system and comparing the results with the classical solvent extraction methodology [20].

3. Results and discussion

The flow cell was designed in order to allow packing the resin beads in the optical path, establishing a resin mini-column in it. Thus, one side of an optical fiber cable was linked at the light source and the other side was fixed inside the optical path of the flow cell, in such a way that the resulting space between the cable and the flow cell walls was lower than the bead diameter. Thus, the beads were retained inside the optical path, whereas the liquid solutions could flow away (Fig. 1). The spent beads were discarded aspirating them back to the holding coil, and directing the suspension to the waste. This strategy permits to pack the beads and to making measures of the absorbance without moves any part of the optical system. Furthermore, it is possible to couple the fiber optical cable at different heights in the optical path, thus obtaining different optical path lengths.

The signal of Hg–Dithizone complex was recorded as the difference between the absorption of the beads on 500 and 620 nm, because Dithizone is partially retained by the beads and the excess of reagent absorbs in both spectrum regions (baseline ca. 0.1).

The flow rate that the sample flows though the resin mini-column was an important parameter only for copper ions retention, probably because the mercury ions presents high affinity by the Chelex-100 beads and its absorption is very fast. Variations in the sample flow rate from 10 to 50 $\mu l\,s^{-1}$ produced only a slight decrease on mercury signals (Fig. 3) but for copper ions, this parameter was very important, as the sensitivity decreased when the sample flow rate was increased (Fig. 3). In this way, the sample flow rate was selected as 10 and 20 $\mu l\,s^{-1}$ for copper and mercury determinations, respectively; aiding to attain the better compromise between analytical frequency, sensitivity and selectivity as at sample flow rate of 10 $\mu l\,s^{-1}$ it was possible to obtain a

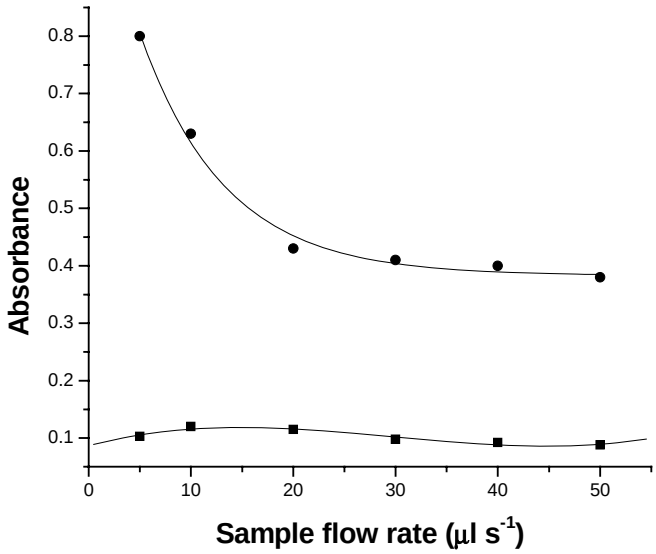

Fig. 3. Effect of sample flow rate in the Hg(II) and Cu(II) analytical signals. The curve was obtained processing 500 μl of a 50 μg l^{-1} Hg(II) and 100 μl of a 500 μg l^{-1} Cu(II) solutions at 5, 10, 20, 30, 40, and 50 μl s^{-1}. Dithizone concentration was fixed as 0.01% (w/v) plus 2.5% (m/v) NH_3; the reagent injected volume and flow rate were 25 μl and10 μl s^{-1}, respectively. APDC concentration was fixed as 0.1% (w/v); the reagent injected volume and flow rate were 100 μl and 40 μl s^{-1}, respectively.

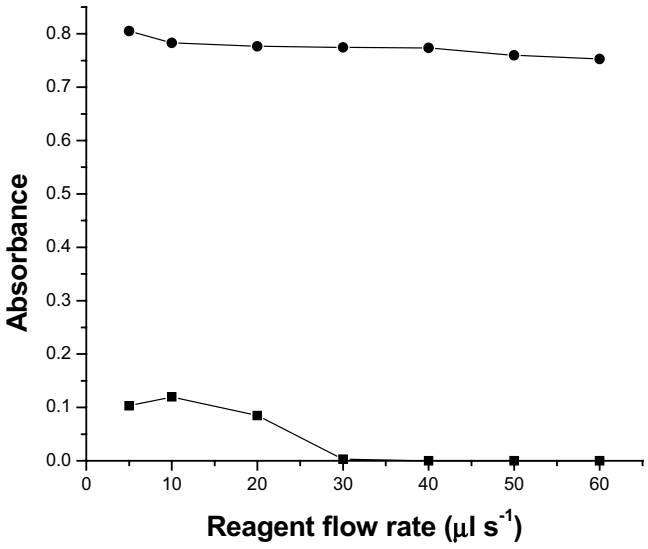

Fig. 4. Effect of Dithizone and APDC flow rate in the analytical signals. The curves were obtained processing 500 μl of a 50 μg l^{-1} Hg(II) solution at 20 μl s^{-1} and 100 μl of a 500 μg l^{-1} Cu(II) solution at 10 μl s^{-1}. The reagent volumes were fixed as 25 and 200 μl mercury and copper determinations, respectively and the flow rates were varied between 5 and 60 μl s^{-1}.

significant analytical signal to copper ions without lost so much time in the concentration step and, at 20 μl s^{-1} it was possible to eliminated the maximum amount of copper ions, that at high concentrations could be an interferant in the mercury determination; without decrease the analytical signal to mercury. When the sample flow rate was 10 μl s^{-1} ca. 80% of the copper ions present in the sample were retained by the resin, whereas to sample flow rate of 20 μl s^{-1} ca. 54% of the copper ions of the sample were retained by the solid phase, which together with the iminodiacetic group of the resin helped to minimizes the interference of copper ions on the mercury determinations when the copper ions content in the sample were higher than 300 μg l^{-1}. It should be emphasised that mercury ions do not interfere in the copper determination, as the APDC reagent does not form coloured complex with Hg(II) ions and the method is almost specific for copper ions. The proposed method is free of interferences of others metallic ions because the concentration step is done in acid pH and under this condition the iminodiacetic group (Chelex functional group) are in the acid form (protoneted) and there are less available electrons to be donate to metallic ions and then, to form the complexes thus, only metal ions that present high affinity by the Chelex-100 resin (Hg(II) and Cu(II)) can be absorbed on the solid phase. Other effect that contributes to increase the selectivity of the method is the absorption kinetic; at flow rate of 10 and 20 μl s^{-1} there is not sufficient time to other metallic ions be absorbed by the solid phase. The same effects were observed by Pai [25], who demonstrated that the absorption of metallic ions by Chelex-100 is extremely dependent of the pH and flow rate and; showing that several metallic ions can be concentrated in the resin only in pH values higher than 6.0.

The effect of the reagents flow rates on the analytical signals showed us an inverse tendency when compared to the sample flow rate. Here, the flow rate of copper reagent (APDC) is not critical, whereas the flow rate of the mercury reagent (Dithizone) is a very important parameter (Fig. 4). These surprising results were probably associated with the fact that reagents flow rates determine the reaction time intervals among the reagents and the metallic ions. This means that at high reagents flow rates the reaction time is low and, at lower reagents flow rates the reaction time is high. As Hg(II) ions are more strongly retained on the beads surface than Cu(II), it is possible to understand why the Dithizone flow rate is more critical than APDC flow rate. Probably the mercury ions retained on the beads surface need a high time interval to react with the Dithizone due to its strong bound with the beads surface which increases the activation energy and consequently reaction rate.

It was observed a decrease in the analytical signal for mercury determination when the Dithizone flow rate was increased from 5 to 30 μl s^{-1} (Fig. 4). For Dithizone flow rates higher than 20 μl s^{-1}, the analytical signals for mercury were not reproducible and, when this value was superior to 30 μl s^{-1}, no analytical signal was observed, probably because there was not sufficient time for colorimetric reaction development. Considering these results, the Dithizone flow rate was fixed at 10 μl s^{-1} for mercury determination. For copper determination, a small variation in the analytical signal was observed when the APDC flow rate was varied from

10 up to 50 μl s^{-1} (Fig. 4) demonstrating that this parameter is not very critical. In this way, 40 μl s^{-1} was elected as the better APDC flow rate for copper determination.

For mercury determination it was verified that there was a compromise between Dithizone concentration and/or Dithizone selected volume and the analytical signal; probably this effect is due to the high affinity of the mercury ions by the Dithizone reagent, as the same effect is not observed in the copper determination. For high concentrations or high selected volumes of Dithizone, the reagent leads to low analytical signals, because partial elution of the Hg–Dithizone complexes from the resin occurred. This effect is associated with the fact that the formed Hg–Dithizone complex is not charged. In this way, the formed complex displaces from the inimodiacetic functional group (Chelex-100) and should be absorbed on the matrix of the solid phase (crosslinkage of styrene divinylbenzene). The presence of high concentrations of Dithizone can saturate the non-functionalised surface of the Chelex-100 beads and thus, the elution of the Hg–Dithizone complex occurs. In this way, to avoid the lost of the formed complex and the consequent error in the mercury determination the concentration of Dithizone reagent should be carefully controlled. On the other hand, if low volumes or low concentrations of the Dithizone reagent are introduced in the flow system, the sensitivity become poor, because low reagent volumes leads to a low interaction time interval between Dithizone and the Hg(II) ions. In situations of low Dithizone concentrations, the reagent amount was not sufficient to reacts with the Hg(II) ions. When 0.0025% (w/v) Dithizone was used, a linear increase of the recorded absorbance was observed as a function of the sample injected volume (from 25 to 100 μl). For higher reagent volumes (>100 μl), the analytical signals decreased due to elution of the Hg–Dithizone complexes. Similar results were obtained when the Dithizone solution was 0.0050% (w/v). However, the maximum absorbance signal was obtained for 50 μl of the reagent solution. When the reagent concentration solution was fixed at 0.0100% (w/v), a selected volume of 50 μl of the reagent solution led to the higher signal, but the measured absorbance was lower than those obtained for more diluted reagent solutions. Considering the above observations, 50 μl of the Dithizone at 0.0050% (w/v) was injected in the flow system.

It was verified that the presence of NH_3 in the Dithizone reagent could improve the analytical signal as NH_3 neutralizes the positive charged imino group of the Chelex-100 beads and could reacts with the proton of the Dithizone reagent. Thus, Dithizone could not be absorbed by the positive charged imino group of the solid phase and, at the same time Dithizone chelating groups became stronger as they were deprotonated, consequently the reaction with mercury ions was faster. For concentrations of NH_3 from 0.625 to 2.5% (w/v), the analytical signal is improved, while at higher NH_3 concentrations a slight decrease in the recorded signal was observed. Thus, the NH_3 concentration was fixed at 2.5% (w/v). As the affinity of the copper ions by the beads is not so high as for mercury ions, 200 μl of APDC 0.1% (w/v) was sufficient to obtain a quantitative analytical signal, this effect was observed elsewhere [13].

The resin suspension volume was selected considering the ideal ion capacity for the selected analytical range, the removal resin facility, and the reproducibility in the amount of selected beads. When the selected resin suspension volume was lower than 20 μl, the reproducibility was lost, as the amount of the selected beads was not a representative sample of the original suspension and, for volumes higher than 50 μl, a high light absorption by the beads occurred, which limited the applicability of the method. In this way, a 40 μl resin suspension volume was selected (corresponds to 1.0 mg of beads), giving an exchange system capacity of 60 μg of Hg(II) or 60 ml of a 1 mg l^{-1} Hg(II) solution in the mercury determination, or 16 μg of Cu(II) or 16 ml of a 1 mg l^{-1} Cu(II) solution, which is sufficient for mercury and copper determinations in water samples. The renew of the mini-column of the Chelex-100 is essential to avoid lost in sensitivity and selectivity as the mercury ions presents high affinity by solid phase and cannot be easy eluted to regenerated the beads (relative affinity by the Chelex-100 resin: Cu(II) = 126 and Hg(II) = 1060).

The selected sample volume for mercury determination was fixed at 1000 μl in order to attain a linear analytical curve between 2.5 and 30 μg l^{-1} of mercury ($A = 0.0052[Hg]+0.1028$, from 2.5 up to 30 μg l^{-1}, $R^2 = 0.995$), whereas for copper determination, the sample selected volume was 100 μl which provides a linear analytical curve 5.0 up to 500 μg l^{-1} of Cu(II) ($A = 0.00081[Cu]+0.058$, from 5.0 up to 500 μg l^{-1}, $R^2 = 0.996$). These parameters were not critical, and these figures of merits can be ranged for mercury and copper determinations in samples with high or low metal ions contents, for this task, the only change is ranged the sample volume [13].

Concerning potential interferants, only metal ions that can be retained by the Chelex-100 resin in 0.014 mol l^{-1} HNO_3 medium were evaluated as after the addition of the HNO_3 as preservative agent the sample become acid. Considering that the Chelex-100 resin is considered an EDTA in the solid phase, the resin is a styrene divinylbenzene polimer with an iminodiacetic functional group; at pH 2 the resin absorbs only Hg(II) and Cu(II) ions. In this way, the possible interferences are: Hg(II) in the copper determination and the Cu(II) in the mercury determination. The first possibility does not occur as the APDC forms coloured complex only with the copper ions, while the APDC–Hg complex absorbs at the UV spectrum region. In this way, although the APDC cannot be considered a selective reagent, the copper determination in the present proposed bead injection procedure can be considered as a specific method. The second possibility of interference is the presence of copper ions during the mercury determination; here the interference is avoided because of two factor, first is the high affinity of the Cu(II) ions by the resin (the reaction between Dithizone and Hg(II) ions is practically specific in the presence of iminodiacetic

Table 2
Sequential determination of total mercury and copper in river-water samples by the proposed method (BI) and by the tradicional solvent extraction (SE) [20]

Sample	Hg(II) BI	Cu(II) BI	Hg(II) SE	Cu(II) SE	Rec1	Rec2
1	4.50 ± 0.7	25.4 ± 0.8	5.0 ± 0.9	27.6 ± 1	105.0	108.0
2	2.33 ± 0.3	19.3 ± 0.6	2.1 ± 0.5	18.9 ± 0.7	93.0	97.5
3	5.80 ± 0.6	34.8 ± 0.7	5.3 ± 0.8	36.1 ± 1	103.1	96.8
4	6.20 ± 0.7	39.3 ± 0.8	6.7 ± 0.9	37.8 ± 1	94.8	104.0
5	–	9.70 ± 0.5	–	8.2 ± 0.6	103.0	96.0
6	3.70 ± 0.4	17.5 ± 0.6	4.1 ± 0.6	18.7 ± 0.8	104.9	107.0

Hg(II) and Cu(II) correspond to the results obtained by the proposed method ($\mu g\,l^{-1}$), and Rec1 and Rec2 to the recovery data (%) for mercury and copper, respectively.

acid) and second because increasing the flow rate of the sample concentration for mercury determination, making sample concentration step at $20\,\mu l\,s^{-1}$ instead of $10\,\mu l\,s^{-1}$, the amount of copper ions retained on the beads surface decrease. If the copper concentration exceeds $300\,\mu g\,l^{-1}$, the interference can be circumvented making to flows 50 μl of a $0.020\,mol\,l^{-1}$ HNO_3 solution through the resin mini-column just before Dithizone reagent injection as, in this situation, the copper ions can be eluted partially while the mercury ions stay on the bead surface.

Remarkably stability and robustness were observed when the proposed system was applied to mercury and copper determinations in water samples. The sequential injection procedure yielded precise results (R.S.D. $\leq$ 9.4 and 5.2% for mercury and copper, respectively). Baseline drift was not observed during long operating periods.

The proposed sequential injection system was able to run about 20 mercury measurements per hour or 45 copper measurements per hour, consuming only 1.25 μg dithizone, 0.20 mg APDC and 1.0 mg Chelex 100 resin per measurement. The analytical ranges were 2.5 up to $30.0\,\mu g\,l^{-1}$ and 5.0 up to $500.0\,\mu g\,l^{-1}$ for Hg(II) and Cu(II) determinations, respectively. These values can be varied increasing or decreasing the selected sample volume. The results are comparable with the classical methods and the recovery tests within 93.0 and 108% were obtained for the river water samples (Table 2). For the above-selected conditions, the detection limit was estimated to be 0.25 and $0.63\,\mu g\,l^{-1}$ for mercury and copper ions, respectively. Again, the analytical range can be change varying the sample-injection volume.

4. Conclusion

The adsorption of Hg(II) and Cu(II) ions in the Chelex-100 resin can be utilized for spectrophotometric sequential bead injection determination of both metal ions with selectivity and sensitivity comparable to the modern analytical techniques as atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry. The advantage of the present method is that the robust and sensitive colorimetric reagents as Dithizone and APDC are utilized in connection with modern extraction methodologies where the hazardous organic solvents are not used, culminating with specific method to Cu(II) determination and a high selectivity method to Hg(II) ions. In addition, the present work demonstrated that the old spectrophotometry is not old fashioned and yet can be utilized in analytical chemistry with high sensitivity, selectivity and exploiting green chemistry.

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