Cloud Point Extraction As a Preconcentration Step for Flame Atomic Absorption Spectrometric Determination of Pb

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INTRODUCTION

Lead (Pb) is an environmental toxic element that may deleteriously affect the nervous, hematopoietic, skeletal, renal, endocrine, and reproductive systems. Environmental lead pollution and its consequent health effects, particularly in children, are still a worldwide problem even after adopting so many preventive measures. The main sources of Pb for humans are food and water. Children are more susceptible to the effects of lead because they absorb lead more readily than adults (1,2). Lead from environmental pollution is not carcinogenic, but even a low-dose Pb exposure has shown to have detrimental and long-lasting effects on the renal, hemopoietic, and nervous systems (3).

Since the Pb levels in urine, hair, water, and tissue are very low, their direct determination is not always possible and a pre-concentration step is necessary. For this purpose, various pre-concentration methods have been developed (4) which include solid phase extraction (5-9), co-precipitation (10-11), atom trap systems (12-14), flow injection-hydride generation technique for infrared spectrometry (15), solvent extraction (16-17), and cloud point extraction (18-24). Co-precipitation is a separation/enrichment technique for trace heavy metal ions. However, this process is slow and samples sometimes have to be kept overnight for

ABSTRACT

Cloud point extraction was employed as a pre-concentration method for the determination of trace amounts of lead (Pb) after formation of a complex with (4-(2-pyridyl-azo)-resorcinol) (PAR) and later analysis by flame atomic absorption spectrometry (FAAS) using polyoxyethylene-7.5 octylphenyl ether (Triton® X-114) as surfactant. After phase separation, based on the cloud point separation of the mixture, the surfactant-rich phase was diluted with 1.0 mol L⁻¹ HNO₃ in methanol. The parameters affecting the separation of the phases and the detection process were optimized. Under the optimum experimental conditions [i.e., pH 9.0, 2.0⁻⁴ L⁻¹ of PAR, 0.1% (w/v) Triton X-114] the detection limits were 2.1 and 1.6 ng mL⁻¹ for 30.0 and 40.0 mL solutions, respectively. The sensitivity of FAAS was improved by 31.6- and 43.7-fold for 30.0 and 40.0 mL of initial volumes, respectively. The developed method was applied to the determination of lead in water samples with satisfactory results. The method was validated by employing certified wastewater CWW-TM-D and spiked/fortified water NWTM-15.

complete co-precipitation. Solvent extraction has been one of the most extensively studied and widely applied method in pre-concentration and separation procedures for the determination of trace elements due to its simplicity, convenience, wide scope, etc. Although this procedure is operated in batch mode, it is time-con-

suming and produces large amounts of potentially toxic organic solvents as waste (4). The use of micellar systems such as cloud point extraction (CPE) has attracted considerable attention in the last few years mainly because it is in agreement with the "green chemistry" principles (25).

Cloud point extraction has been employed in analytical chemistry to separate and pre-concentrate organic compounds (26-28) and metal ions (25, 29-31). When aqueous solutions of nonionic or amphoteric surfactants above their critical micellar concentration (CMC) are heated at a characteristic temperature (called cloud point temperature), the solution containing the surfactant becomes turbid and separates into a surfactant-rich phase (very small viscous volume) and the remaining larger volume (bulk amount) into the diluted aqueous solution with the surfactant concentration, which is approximately equal to its critical micellar concentration. So, a high pre-concentration factor can be obtained (4). In comparison with the traditional organic liquid-liquid extraction, CPE requires a very small amount of relatively non-flammable and non-volatile surfactants. For this purpose, different nonionic surfactants such as Triton® X-100, Triton® X-114, PONPE 7.5, Tween 80, and doubly ionic surfactants such as ammonioethylsulfates, ammoniopropylsulfates, ammoniopropansulfanates, phosphobetaine, and dimethylalkylphosphine oxides have been used (28). This method permits the design of extraction schemes that are lower in toxicity and offer high efficiency.

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According to a literature survey, the combination of PAR and Triton X-114 for the pre-concentration of Pb has not been applied before with cloud point extraction. In this work, a method for the cloud point pre-concentration coupled with FAAS for Pb, using (4-(2-pyridyl)-azo-resorcinol) (PAR) as complexing reagent and Triton X-114 as surfactant, was developed. The results showed that Pb was determined successfully with satisfactory recoveries and precision.

EXPERIMENTAL

Instrumentation

An ATI UNICAM Model 929 flame atomic absorption spectrophotometer (equipped with an ATI UNICAM hollow cathode lamp as the radiation source) was used for the determination of lead (ATI UNICAM, USA). The FAAS experimental conditions for Pb are given in Table I. The pH was measured with a Mettler Toledo pH meter. A thermostated bath maintained at the desired temperature was used for the experiments and phase separation was assisted using a centrifuge in 50.0 mL calibrated centrifuge tubes.

Reagents and Solutions

The non-ionic surfactant Triton X-114 (Sigma Aldrich, Germany) was used without further purification. Stock standard solutions of Pb at a concentration of 1000 $\mu\text{g mL}^{-1}$ were prepared from its nitrate salt. Working standard solutions were obtained by appropriate dilutions of the stock standard solutions.

A 1.10^{-2} mol L^{-1} solution of PAR (Fluka, Germany) was prepared by dissolving appropriate amounts of these reagents in ethanol (Merck, Germany) which were kept in a refrigerator (4°C).

All other reagents were of analytical reagent grade and during all analytical work, doubly distilled water was used. The pipettes and vessels used for trace analysis were kept in dilute nitric acid at least overnight and then subsequently washed with distilled water.

Na(I), K(I), Ca(II), and Mg(II) solutions were prepared from their stock solutions of 1000 $\mu\text{g mL}^{-1}$ and common anions, such as Cl^{-} , NO_3^{-} , CO_3^{-2} , PO_4^{-3} , and SO_4^{-2} , were prepared from their sodium salts.

CWW-TM-D and NWTM-15 were supplied from High Purity Standard, USA and LGC Standards (Middlesex, United Kingdom).

Samples

Water samples were obtained from three different sources: (a) Tap water from the laboratory was collected in 5.0-L bottles after first letting it flow for 5.0 minutes. (b) Tigris River water was collected at five different parts of the river in 1.0-L glass bottles and brought to the laboratory. Then, 0.25 mL of concentrated HNO_3 was added to each bottle, and mixed before filtering with a $0.45\text{-}\mu\text{m}$ membrane filter. (c) Effluent water was collected from an industrial region in Diyarbakır (Gözü-Diyarbakır) and sampled like the river water in 1.0-L bottles. All of the samples were

immediately transferred to the laboratory and subjected to the CPE procedure. The Pb concentrations in the samples were determined by FAAS.

Certified Reference Samples

1.0 mL of certified wastewater CWW-TM-D sample was diluted to 40.0 mL with distilled water before applying the CPE method. The CPE method was directly applied to the spiked/fortified water NWTM-15 after pH adjustment.

Procedure

For the cloud point extraction, 30.0-mL aliquots of the cold solutions containing the Pb at the concentration of 20.0 ng mL^{-1} , Triton X-114 0.2 % (w/v) and 1.10^{-4} mol L^{-1} PAR, adjusted to a desired pH, were kept for 15 minutes in a thermostatic bath at 45°C . The separation of the phases was accelerated by centrifuging at 5500 rpm for 10 minutes. After cooling in an ice-bath, the surfactant-rich phases become viscous. Then, the aqueous phase was separated by inverting the tube. Subsequently, to decrease the viscosity of the surfactant-rich phase, 1.0 mL of 1.0 mol L^{-1} HNO_3 in methanol was added. Blanks were prepared with the same reagents, without the samples, undergoing an identical process. The pre-concentrated solutions were introduced into the flame by conventional aspiration.

RESULTS AND DISCUSSION

Effect of pH

The pre-concentration of metal ions by the cloud point method involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of the surfactant-rich phase, thus obtaining the desired pre-concentration. The extraction efficiency depends on the pH at which the complex formation is carried out. Thus, the effect of the pH on

TABLE I
Instrumental Operating Parameters for FAAS

Parameters	Pb
Wavelength	217.0 nm
Hollow cathode lamp current	9.5 mA
Acetylene flow rate	0.5 L min^{-1}
Air flow rate	4.0 L min^{-1}
Slit width	0.5 nm

the extraction of Pb was investigated in the 1.0–12.0 pH range and the results are shown in Figure 1. It was found that the atomic absorbance reached maximum at about pH 9.0, at which point maximum extraction efficiency was obtained. For this study, pH 9.0 was optimized as the working pH. In addition, the pH of all of the solutions was buffered at pH 9.0 by using a borate buffer.

Effect of PAR Concentration

The concentration of a chelating reagent has a remarkable effect on the extraction efficiency. While all other experimental parameters are constant, the effect of the optimum concentration of the chelating reagent on the recovery was studied and the results were given in Figure 2. It can be seen that maxi-

mum recovery was obtained at 2.10^{-4} mol L⁻¹ PAR; as a result, this concentration was chosen as the optimum concentration of the chelating reagents.

Effect of Triton X-114 Concentration

In comparison to other surfactants, Triton X-114 provides high density of the surfactant-rich phase as well as its appropriate cloud point temperature (23–25 °C) and was used for performing CPE. The variations in the analytical signals as a function of the concentration of Triton X-114 in the range of 0.025–0.25% (w/v) were investigated. Quantitative extraction was observed when the Triton X-114 concentration was > 0.05 %. Figure 3 highlights the differences observed in the analytical signals at different

concentrations above 0.05%. The signals were decreased owing to the increase in volume and the viscosity of the surfactant phase. An amount of 0.1% (w/v) Triton X-114 was selected to achieve the greatest analytical signals, thereby the highest extraction efficiency.

Effect of Equilibration Temperature and Centrifugation Time

To optimize the method, it was also necessary to examine the effect of temperature on cloud point extraction. As a compromise between completion of extraction and efficient separation of the phases, the lowest possible equilibration temperature and the shortest incubation time were employed. The results illustrated in Figure 4 show excellent recovery for the equilibration temperature from 40 to 50 °C;

Fig. 1. Influence of the pH on the recovery of Pb. Conditions: 30.0 mL of cold aqueous solution contain 20.0 ng mL⁻¹ Pb, 1.10^{-4} mol L⁻¹ PAR, 0.2% (w/v) Triton X-114 at 45 °C.

Fig. 2. Influence of the PAR concentration on the recovery of Pb. Conditions: 30.0 mL of cold aqueous solution contain 20.0 ng mL⁻¹ Pb, pH 9.0, 0.2% (w/v) Triton X-114 at 45 °C.

Fig. 3. Variations of the recovery of the Pb with Triton X-114 concentrations. Conditions: 30.0 mL of cold aqueous solution, 20.0 ng mL⁻¹ Pb, pH 9.0, 2.10^{-4} mol L⁻¹ PAR at 45 °C.

Fig. 4. Effect of the equilibration temperature on the analytical signal. Conditions: 30.0 mL of cold aqueous solution contain 20.0 ng mL⁻¹ Pb, pH 9.0, 2.10^{-4} mol L⁻¹ PAR, 0.1% Triton X-114 at 45 °C.

and 45 °C was chosen for the equilibration temperature.

The effect of centrifugation time on extraction efficiency was studied in the time range of 5.0–25.0 minutes from 3000 to 6000 rpm. A centrifugation time of 10 minutes at 5000 rpm was selected for the entire procedure since analyte extraction in this time period is almost quantitative.

Effect of Diverse Ions

The interference of foreign ions on the determination of lead was investigated by adding a known quantity of the desired ion to a 40.0-mL aliquot of aqueous solution containing 20.0 ng mL⁻¹ of lead. The tolerance limit of foreign ions was taken as the value that caused an error of not more than ±5% in the absorbance. No interference was found from common cations, such as Na(I), K(I), Ca(II), and Mg(II), and common anions, such as Cl⁻, NO₃⁻, CO₃⁻², PO₄⁻³, and SO₄⁻², over the investigation range, which were present at concentrations of 5000 times that of lead. Similarly, the other cations tested did not cause any serious interference, even when present at concentrations 500 times that of lead.

Characteristics of the Method

A calibration curve was constructed by pre-concentrating 30.0 and 40.0 mL of solutions in the

presence of 0.1% (w/v) Triton X-114 at pH 9.0 and under the other experimental conditions specified in the procedure. The samples were introduced into the flame following the addition of 1.0 mL of 1.0 mol L⁻¹ HNO₃ in methanol. In this case, linear relationships between the absorbance measured and the concentration of the metal placed in solutions were obtained. Table II lists the parameters of the calibration curves, the relative standard deviations obtained for 3 replicates subjected to the complete procedure, and the detection limits. Table II also gives the calibrations obtained with standard solutions of Pb not subjected to the pre-concentration step.

The linear calibration range of the method was obtained as 10.0–30.0 ng mL⁻¹ for 30.0 mL of initial solutions and 5.0–25.0 ng mL⁻¹ for 40.0 mL of initial solutions. The limit of detection (LOD) was found to be 2.1 ng mL⁻¹ and 1.6 ng mL⁻¹ for 30 and 40 mL of solutions, respectively. The enhancement factor was calculated as 31.6 and 43.7 for 30.0 and 40.0 mL of solutions, respectively.

The LOD values obtained from our study were compared with studies published in the literature. 1-phenylthiosemicarbazide (32), ammonium pyrrolidinedithiocarbamate (APDC) and diethyldithiocarbamate (DDTC) (33),

3-(1-(1-HIndol-3-Yl)-3-phenylallyl)-1H-indole (34), bis((1H-benzo(d)imidazol-2yl)ethyl) sulfane (35), brilliant cresyl blue (18), APDC (36), and 3-((4-bromophenyl) (1-H-inden-3-yl)methyl)-1 H-indene (37) were used as complexation reagent for Pb. The LOD values were in the range of 1.14–7.5 ng mL⁻¹ (18, 32–37). Therefore, it could be said that our method gave comparable LODs and low levels of Pb can be determined by this method.

Application of Method to Water Samples

Before application of this method to real samples, it was applied to certified reference water samples. The accuracy of the method was tested using certified reference materials wastewater CWW-TM-D and spiked/fortified water NWTM-15. 40.0 mL of certified water samples (CWW-TM-D diluted by 40.0 times) were subjected to the CPE method to determine its Pb content. No digestion procedure was applied to the samples. The results showed that the found values agreed with the certified values. The relative standard deviations were found to be lower than 2.1% (see Table III).

The Pb concentrations in several liquid samples were determined by the developed CPE procedure. When the samples were collected, they were immediately filtered

TABLE II
Analytical Characteristic of the Method

Conditions	Concentration Range (ng mL ⁻¹)	Slope ^a	Intercept	r ²	(%) RSD	LOD ^b (n=3)	E ^c
Without pre-concentration	250.0-1000.0	0.038	1.0	0.9974	2.4 (750.0 ng mL ⁻¹)	65	1
With pre-concentration ^d	10.0-50.0	1.20	-0.4	0.9978	3.2 (25.0 ng mL ⁻¹)	2.1	31.6
With pre-concentration ^e	5.0-25.0	1.66	-0.3	0.9938	2.8 (15.0 ng mL ⁻¹)	1.6	43.7

^a Linear calibration plots were constructed by considering Pb concentration versus related absorbance x 1000.

^b Limit of detection defined as the ratio of three times of the standard deviations of the lowest concentrations of linear range after CPE procedure to slope of calibration plot.

^c Enhancement factor was calculated from slope ratio of linear calibration plots of each of methods.

^d For 30.0 mL of standard solutions.

^e For 40.0 mL of standard solutions.

TABLE III
Determination of Lead in
Certified Reference Samples

Reference Sample	Certified	Measured	RSD (%)
CWW-TM-D ^a	1.0 µg mL ⁻¹	0.96±0.02 µg mL ⁻¹	2.1
NWTM-15	11.8 ng mL ⁻¹	11.3±0.1 ng mL ⁻¹	0.9

^a It was diluted by 40.0 times.

TABLE IV
Determination of Lead in
Liquid Food Samples

Sample	Added (ng mL ⁻¹)	Found (ng mL ⁻¹)	RSD (%) (n=3)
Tap Water	-	-	-
	10.0	10.2±0.2	2.0
Tigris River Water	-	-	-
	10.0	9.7±0.2	2.1
Effluent Water	-	8.3±0.2	2.4
	10.0	17.8±0.3	1.7

through a membrane filter (pore size: 0.45 µm) to remove any suspended matter and stored in polyethylene bottles. Table IV lists the results of the analyses of the original sample solutions by the proposed procedure. In addition, the samples were spiked with known amounts of Pb and recovered quantitatively. The obtained results (Table IV) indicate that the proposed method is applicable to the analysis of water samples. These results demonstrate the utility of the proposed method for the pre-concentration and measurement of trace amounts of Pb without interferences.

CONCLUSION

The developed method is based on the complexation of lead (Pb) with 4-(2-pyridyl-azo)-resorcinol (PAR) and extraction of the complex with non-ionic surfactant Triton X-114 by cloud point extraction. The Pb content in solution was determined by flame atomic absorption spectrometry (FAAS). The sensitivity of FAAS was improved by applying the cloud point extraction (CPE) method. The improvement factors were 31.6- and 43.7- fold for 30.0 and 40.0 mL of initial volumes, respectively. The use of PAR as a ligand for Pb was utilized for the determination of Pb in water samples. The proposed method allowed the determination of Pb at ng mL⁻¹ levels. Thus, it represents a promising

approach in the monitoring of Pb in different samples at low cost with simplicity and efficiency. The proposed CPE method gives low LOD and RSD values and offers solvent-free extraction of Pb from its initial matrix.

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