Vortex-assisted Dispersive Liquid-Liquid Microextraction of Pb(II) as 2-hydroxypyridine-3-carboxylic Acid Chelates From Food and Water Samples Prior to Flame Atomic Absorption Spectrometric Determination

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ABSTRACT

A vortex-assisted dispersive liquid-liquid microextraction method for the preconcentration and separation of lead(II) as 2-hydroxypyridine-3-carboxylic acid chelates is presented. CCl_4 as the extraction solvent and ethanol as the dispersive solvent were used. The analytical parameters optimized included pH, amount of 2-hydroxypyridine-3-carboxylic acid, type and volume of extraction solvent, type and volume of dispersive solvent, sample volume, etc., which affect the extraction efficiency of lead(II). The influence of some matrix ions was also investigated. The detection limit for lead(II) was 8.30 μg L^{-1} for liquid samples and 0.49 μg g^{-1} for solid samples. The preconcentration factor of the method was 30. The validation of the method was checked by the analysis of the certified reference materials TMDA-64.2, TMDA 53.3, and SPS-WW2 Wastewater and GBW07425 soil, BCR-146 R Sewage Sludge, HR-1 Harbour Sediment, and DC73349 Bush Branches and Leaves. The proposed microextraction method was applied to the determination of lead in some natural water, food, soil, and sediment samples.

INTRODUCTION

Investigation of the levels of transition metal ions in the environment is very important (1-4) due to their negative and harmful influence on humans, plants, and animals. Elements such as cobalt, copper, and zinc (5-7) are necessary at trace levels, while lead, cadmium, and mercury have harmful effects (8-10). The environment is often contaminated simultaneously by more than one heavy metal (11-13). Humans are exposed to lead from air and food in roughly equal proportion (12). Lead contamination is also found in wells, and is due to improper protection and industrial activities (13). Although the amount of lead in petrol has dramatically decreased, the metal should not be used in food containers, and lead-based paints should be completely abandoned. In particular, the public should be made aware of glazed food containers since lead may leach into the food (12, 14-16). Cigarettes are another important source for lead exposure to humans (18-20). The accurate and precise determination of lead at ultratrace levels in environmental samples, including food and water, is a very important part of analytical chemistry studies (21-25).

Atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) are the most commonly used instrumental techniques for the determination of the trace level analysis of environmental samples (26-31). However, a separation-preconcentration step prior to analysis is necessary due to matrix effects since the analyte levels are often lower than the detection limits of the instrument (32-34). Studies for improving the detection limits and the accuracy of an analysis for samples containing large amounts of highly saline matrices have been reported using various kinds of techniques, including cloud point extraction, solvent extraction, and solid phase extraction (35-39).

In order to reach the rules of green chemistry, different authors have proposed microextraction techniques by miniaturizing the separation-preconcentration techniques using reduced amounts of hazardous solvents and waste (40-42). These solutions will also reduce major spectral and physical interferences and, therefore, microextration techniques will lead to improvement in sensitivity of the instrumental detection systems. The method of dispersive liquid–liquid microextraction (DLLME) has an important place in this area. The benefits of DLLME are simple operation with a high preconcentration factor, accurate results, low cost, environmentally friendly, consumption of small quantities of all the necessary chemical reagents, and rapid detection.

In the present work, a novel vortex-assisted dispersive liquid-liquid microextraction system was established for the preconcentration-separation of lead at ultratrace levels as 2-hydroxypyridine-3-carboxylic acid chelates. The analytical conditions for the quantitative retention of lead ions by the proposed method have been optimized.

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EXPERIMENTAL

Instrumentation

A Model 3110 flame atomic absorption spectrometer (FAAS) (PerkinElmer, Inc., Shelton, CT, USA) was used for the determination of lead (Pb). All of the absorbance values were measured using an air/acetylene flame. A 10-cm long slot-burner head and a Pb hollow cathode lamp were used. The instrumental settings for lead analysis were in accordance with the manufacturer's instructions. The samples were injected into the nebulizer of the instrument by applying the micro-injection technique. An amount of 50 µL of each sample was transferred by syringe to a mini home-made Teflon® funnel which was attached to the nebulizer with capillary tubing and an Eppendorf pipette. The peak height signals were reported.

A Metrohm Model 691 pH meter (Metrohm AG, Switzerland) with a combined glass electrode was used for the pH measurements. An ALC PK 120 model centrifuge (ALC, Buckinghamshire, England) was used for the centrifugation process and a vortex mixer (Wiggen Hauser, Malaysia) for thoroughly mixing the solutions.

Reagents and Solutions

All reagents and chemicals were of analytical grade. All solutions were prepared using a Milli-Q® system for reverse osmosis ultra-pure water with a resistivity of 18.2 $M\Omega \cdot cm^{-1}$ (Millipore Corporation, France). Standard solutions of Pb(II) (1000 mg L^{-1}) were prepared by dissolving nitrate salt (Merck, Darmstadt, Germany) in ultrapure water. The working standard solutions of Pb(II) were obtained by serial dilution of the standard solutions. External calibration standards were not subjected to the separation-preconcentration procedure.

Certified reference materials (CRM) TMDA 53.3 and TMDA 64.2 Water for Trace Elements were supplied by the National Water Research Institute, Environment Canada, Burlington, Canada. CRM SPS-WW2 Wastewater Level 2 was supplied by Spectrapure Standards AS, Oslo, Norway. CRM GBW07425 (GSS-11) Soil (National Research Centre for Certified Reference Materials, China), BCR 146-R Sewage Sludge (the Community Bureau of References, Belgium), HR-1 Harbour River Sediment (National Water Research Institute, Ontario, Canada), and NCS DC73349 Bush Branches and Leaves (National Research Centre for Certified Reference Materials, Beijing, P.R. China) were also used for the accuracy studies.

A solution of 0.05% (w/v) 2-hydroxypyridine-3-carboxylic acid (Sigma-Aldrich, Germany) was prepared using deionized water. The pH values were adjusted by the addition of phosphate buffer solution ($H_2PO_4^-/H_3PO_4$), acetate buffer solution (CH_3COO^-/CH_3COOH), and ammonium buffer solutions (NH_4^+/NH_3).

Microextraction Procedure

An aliquot of 10 mL of the sample solution containing 200 µg L^{-1} Pb(II) at pH 7.0 was placed into a 50-mL conical bottom test tube. Then 5 mL of dihydrogen phosphate/disodium hydrogen phosphate buffer (pH 7) and 150 µL of 2-hydroxypyridine-3-carboxylic acid (0.1 % (w/v)) were added, and the solution was left standing for 5 minutes for complex formation. Next, 150 µL carbon tetrachloride and 1250 µL ethanol were rapidly injected into the sample using a 5.0-mL syringe. The tube was shaken with a vortex stirrer for two minutes. A cloudy solution was thus formed, extracting the Pb(II)-2-hydroxypyridine-3-carboxylic acid complex into the organic phase. Centrifugation at 4000 rpm for six minutes allowed the formation of two well-defined phases. The upper aqueous phase was manually removed with a syringe, and the organic phase was dissolved with 500 µL concentrated HNO_3. Of this solution, 100 µL was introduced into the FAAS nebulizer using a micro-injection system to measure the lead concentration. A blank solution was processed using the same procedure as explained above.

Applications

An aliquot of 0.25 g each of the CRM NCS DC73349 Bush Branches & Leaves and of each food sample was digested with 10 mL nitric acid at 100 °C. The mixture was evaporated to near dryness. Five milliliters of H_2O_2 and 10 mL nitric acid were added, and the sample again evaporated to near dryness. After evaporation, 5 mL of water was added, and the sample mixed. The resulting mixture was filtered through a blue ribbon filter paper, and the procedure described in "Microextraction Procedure" section was applied.

An aliquot of 0.25 g each of GBW07425 (GSS-11) Soil, BCR 146-R Sewage Sludge, HR-1 Harbour River Sediment, and soil and sediment samples from different regions in Turkey were weighed. Each sample was transferred to a Pyrex® tube, and 10 mL of *aqua regia* was added. The sample was transferred to a heating block for 6 hours for complete digestion. The residue was filtered through a blue ribbon filter paper, the filtrate combined with leachate, and diluted with 5 mL of deionized water. Then the procedure described in the "Microextraction Procedure" section was applied.

The method was also applied to CRM TMDA 53.3 Water for Trace Elements, TMDA-64.2 Water for Trace Elements, SPS-WW2 Waste Water Level 2, and water samples

from several different locations in Turkey. The water samples collected in polyethylene bottles were filtered through a cellulose membrane filter (Millipore Corporation, USA) of 0.45 µm pore size. Then, the procedure described in "Microextraction Procedure" section was applied.

RESULTS AND DISCUSSION

Effect of pH on the Microextraction Efficiency of Pb(II)

In extraction studies, the pH of the aqueous solution is one of the main factors for achieving maximum recoveries (43-47). The effect of the pH of the model solutions that contain Pb(II) were investigated at the pH range of 4.0–8.0, and all other conditions were kept constant. The results are shown in Figure 1. The recovery of the lead ions was quantified at the pH 6.5 and 7.0. A pH of 7.0 was selected for subsequent work which was obtained by using 5 mL of dihydrogen phosphate/disodium hydrogen phosphate buffer solution.

Influence of Amounts of 2-hydroxypyridine-3-carboxylic Acid on the Microextraction Efficiency of Pb(II)

The organic chelating agent 2-hydroxypyridine-3-carboxylic acid was selected for the present microextraction system. The effects of the amounts of 2-hydroxypyridine-3-carboxylic acid on the microextraction efficiency of the lead ions were also studied in the range of 0.0–0.3 mg, and the results are shown in Figure 2. The amount of Pb(II) recovered with the present DLLM method was less than 60% without the chelating agent. Lead recovery increased with increasing amounts of chelating agent and reached a quantitative value (>95 %) at 0.1 mg of 2-hydroxypyridine-3-carboxylic acid. The recovery values were quantitative in the range of 0.1–0.2 mg of chelating agent. All other experiments were conducted using 0.15 mg of 2-hydroxypyridine-3-carboxylic acid.

Choice of Extraction Solvent

In order to choose a suitable extraction solvent for maximum extraction efficiencies, different solvents were examined as listed in Table I. Quantitative recoveries (>95 %) were obtained only with carbon tetrachloride.

Different amounts of carbon tetrachloride (CCl_4) were tested ranging from 50–250 µL. The results in Figure 3 show that quantitative recoveries for Pb(II) were obtained in the 100–150 µL range. For all further experiments, 150 µL of carbon tetrachloride was used.

Choice of Dispersive Solvent

Various dispersive solvents were used for the microextraction of Pb(II) in the present study. The results in Table II show that quantitative recoveries were obtained with ethanol. The effect of the volume of ethanol on the recoveries of Pb(II) was studied in the range of 0–1500 µL. The results in Figure 4 indicate that the recoveries were quantitative at 1250 µL of ethanol and this amount was chosen for subsequent work.

Influence of Vortex Time

The influence of vortex time on the recovery of Pb(II) was examined in the range of 2–5 minutes. Quantitative recoveries were obtained in the range of 2–5 minutes, and a vortex time of 2 minutes was used for further work.

TABLE I
Effects of Various Extraction Solvents on the Recoveries of Pb(II) (N=3)

Extraction Solvent	Recovery (%)
Dichloromethane	33±3
Carbon disulfide	30±0
Chloroform	28±3
Carbon tetrachloride	100±0

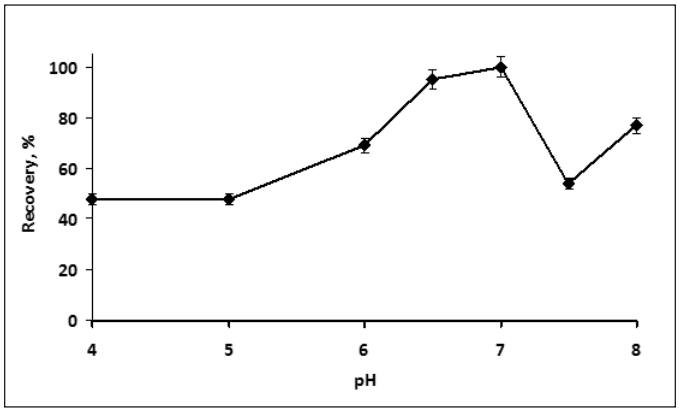

Fig. 1. Effect of pH on the microextraction efficiency of Pb(II) (N=3).

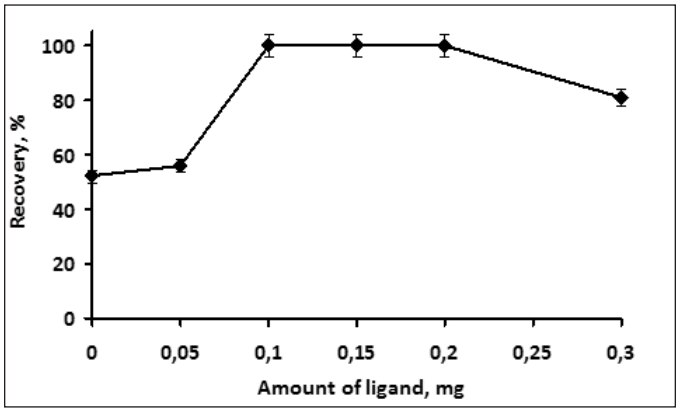

Fig. 2. Effects of ligand amount on the recoveries of Pb(II) (N=3).

Influence of Centrifuge Time on the Recoveries

The effect of centrifugation time on the recoveries of Pb(II) was investigated in the range of 2-10 minutes at 4000 rpm centrifugation speed. The recoveries of Pb(II) were quantitative (>95 %) at 6 minutes. Thus, 4000 rpm for 6 minutes was selected for centrifugation speed and time for further work.

Sample Volume

To obtain a high preconcentration factor (48–55), the volume of the sample is a critical factor. The effects of sample volume on Pb(II) recovery were studied in the range of 10–40 mL. The recovery was quantitative (>95%) with 15 mL. A preconcentration factor of 30 can be achieved when performed with a 15-mL sample volume and a 500-µL final volume.

TABLE II
Effects of Type of Dispersive Solvent on the Recovery of Pb(II) (N=3)

Dispersive Solvent	Recovery (%)
Methanol	22±3
Acetonitrile	41±1
Dimethyl formamide	20±2
Acetone	24±3
Ethanol	100±0

Matrix Effects

The effects of matrix ions in real samples including some 1A, 2A, and transition metals as well as some anions that may positively or negatively influence the absorbance of analyte elements in the FAAS determination (56–63), and the FAAS detection of Pb(II) using the present microextraction system, was also investigated and the results are shown in Table III. The tolerance limit is defined as the ion concentration causing a relative error smaller than ±5% related to the preconcentration and determination of Pb(II). These tolerable levels of matrix ions are suitable for the FAAS detection of analyte elements in real samples including natural water and food samples.

Analytical Performance

The limit of detection (LOD), calculated based on 10 determinations of the standard deviation of the blank, was 8.30 µg L^{-1} for liquid samples. The limit of quantification (LOQ), calculated as 3.3 times the LOD, was 27.39 µg L^{-1} for liquid samples. The detection limit (LOD) and the limit of quantification (LOQ) for lead in solid samples was 0.49 µg g^{-1} and 1.62 µg g^{-1}, respectively. The relative standard deviation (RSD), determined from seven analyses of the standard solution of Pb(II) (0.50 µg), was 5.9%.

TABLE III
Matrix Effects (N=3)

Ions	Added	Concentration (µg/mL)	Recovery (%)
Na^+	$NaNO_3$	5000	98 ±2
K^+	KCl	1000	100±2
Ca^{2+}	$Ca(NO_3)_2 \cdot 4H_2O$	1000	100 ±5
Zn^{2+}	$Zn(NO_3)_2 \cdot 6H_2O$	2.5	95±4
Cr^{3+}	$Cr(NO_3)_3 \cdot 9H_2O$	10	98 ±6
Mg^{2+}	$Mg(NO_3)_2 \cdot 6H_2O$	750	94 ±2
Fe^{3+}	$Fe(NO_3)_3 \cdot 9H_2O$	2.5	96 ±4
Mn^{2+}	$Mn(NO_3)_2 \cdot 4H_2O$	5	94 ±5
SO_4^{2-}	Na_2SO_4	2500	104 ±6
Cl^-	KCl	1000	100±2

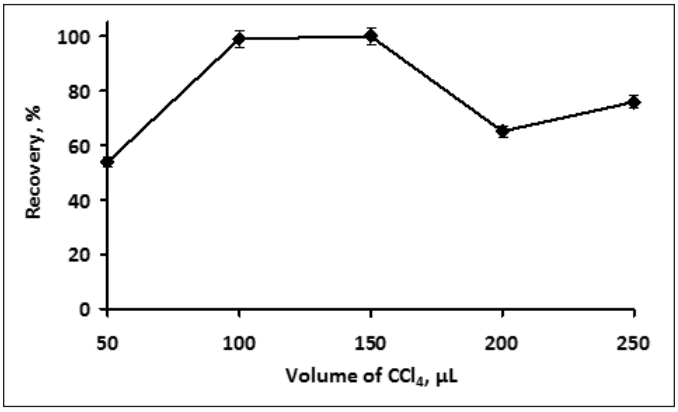

Fig. 3. Influence of volume of CCl4 on the extraction efficiencies of Pb(II) (N=3).

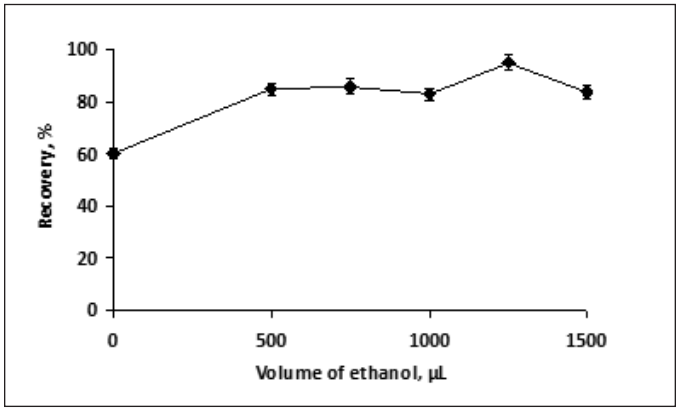

Fig. 4. Effect of ethanol volume on the recoveries of Pb(II) (N=3).

Analysis of Real Samples

The validity of the present procedure was checked by analyzing various certified reference materials. It was found that the results as listed in Table IV are in good agreement with the certified values.

The proposed microextraction method was applied to the FAAS determination of trace lead concentrations in some water and food samples found in Turkey and the results are presented in Table V. The levels of lead in the samples given in Table V were found to be at trace levels in the soil and water samples and at ultratrace levels in the food samples.

CONCLUSION

A novel and simple vortex-assisted dispersive liquid–liquid microextraction (DLLME) technique combined with flame atomic absorption spectrometry (FAAS) is presented for the separation, preconcentration, and determination of lead(II) in environmental samples. Lead(II) was quantitatively recovered at pH 7.0 as 2-hydroxypyridine-3-carboxylic acid complexes. The main two advantages of the presented microextraction procedure are:

(a) The determination of μg L^{-1} and μg g^{-1} levels of lead is possible with flame atomic absorption spectrometry which is low in instrumental cost in comparison to graphite furnace atomic absorption spectrometry.

(b) The present procedure requires lower amounts of solvent to achieve green chemistry. Thus, the DLLME method presented here is environmentally friendly, economical, and safe.

TABLE IV
Application of Present Method to the Determination of Pb(II) Certified Reference Materials (N=5)

Certified Reference Material	Found (μg L^{-1})	Certified (μg L^{-1})	Recovery (%)
TMDA-53.3 Water for Trace Elements	339±22	349	97
TMDA-64.2 Water for Trace Elements	287±20	288	100
SPS-WW2 Wastewater	508±39	500±3	102
	Found (μg L^{-1})	Certified (μg L^{-1})	Recovery (%)
GBW07425 (GSS-11) Soil	24.4±0.2	24.7±1.4	99
BCR-146 R Sewage Sludge	609±19	583±17	104
HR-1 Harbour Sediment	134±2	139	96
DC73349 Bush Branches and Leaves	49±3	47±3	104

TABLE V
Application of Present Method to the Determination of Pb(II) in Wastewater, Food, Soil, and Sediment Samples (N=5)

Samples	Concentration (μg L^{-1})
Wastewater-I from industrial region of Kayseri	Below LOQ
Wastewater-II from industrial region of Kayseri	50±6
Wastewater-III from industrial region of Kayseri	Below LOQ
Wastewater-IV from industrial region of Kayseri	70±2
	Concentration (μg g^{-1})
Cucumber	Below LOQ
Apple	Below LOQ
Cherry	Below LOQ
Bean	Below LOQ
Soil from Kayseri	14.0±1.4
Soil from Fethullah, Yahyali	28.4±2.3
Soil from Kirazli, Yahyali	11.5±0.0
Sediment from Yahyali	11.1±1.4

ACKNOWLEDGMENT

The authors are grateful for the financial support of the Unit of the Scientific Research Project of Erciyes University (Project No. FYL-2014-5067). The authors thank Erkan Yilmaz for his friendship and help in the experimental studies

Received September 28, 2015.

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