Micro-organic Ion-associate Phase Extraction/micro-volume Back-extraction for the Preconcentration and GF-AAS Determination of Cadmium, Nickel and Lead in Environmental Water

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Micro-organic ion-associate phase (IAP) extraction was combined with a micro-volume back-extraction (MVBE) to reduce coexisting components and viscosity in the concentrates. Heavy metals were converted into a complex with 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol in a 40-mL sample solution, and were extracted into ion associates. After centrifugation and discarding the aqueous phase, trace metals were stripped from IAP into a nitric acid solution, followed by GF-AAS determination. Only one vessel was required for 400-fold enrichment. The detection limits ($3\sigma_b$) for Cd, Ni, and Pb were 0.6, 3.7, and 0.8 ng/L, respectively. This method was applied in recovery tests in seawater.

Keywords Ion-associate phase, micro-volume back-extraction, GF-AAS

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Introduction

To date, the separation of analytes from other interfering components during a measurement and a further increase of the sensitivity *via* enrichment are still important, despite an improved sensitivity of analytical instruments. The value for the Pb concentration in WHO guidelines for drinking water quality¹ is a provisional value because the minimum measurable value is greater than the guideline level.

Different preconcentration/separation methods are employed for quantifying trace heavy metals in water, such as liquid-liquid extraction,²⁻⁴ coprecipitation,⁵ solid-phase extraction,^{6,7} cloud point extraction,^{8,9} dispersive liquid-liquid extraction,¹⁰ thermoresponsive polymer-mediated extraction,¹¹ homogeneous liquid-liquid extraction,¹² and enrichment of the membrane filter.¹³ The above-mentioned separation/preconcentration techniques comprise the use of xylene,^{2,4} diisobutylketone,³ lanthanum phosphate,⁵ dependence on equilibration temperature and time,^{8,9} the use of trichloroethylene as an extraction solvent,¹⁰ the application of heat,¹¹ or the use of a fluorine-based surfactant.¹² The metals collected on a membrane filter were determined by energy-dispersive X-ray fluorescence (EDXRF) spectrometry.¹³ These methods are attainable higher enrichment.

IAP extraction¹⁴⁻¹⁸ is a unique technique for separating, enriching, and determining traces in water. This method was already applied for the separation and spectrophotometric determination of ammonia in river water,¹⁴ nitrite in river water and seawater,¹⁵ the HPLC determination of di(2-ethylhexyl)-phthalate in river water,¹⁶ the GF-AAS determination of Cd in

river water and seawater,¹⁷ and the liquid electrode plasma emission spectrometry (LEP-AES) determination of trace metals in waste water.¹⁸

In the present work, to avoid the use of organic solvent for extraction and to reduce coexisting components in the concentrate, IAP extraction was combined with a micro-volume back-extraction technique (MVBE) with acids. Unlike liquid-liquid extraction/MVBE,²⁻⁴ this preconcentration/separation technique does not use any organic solvent. The acids rapidly stripped targeted trace metals from IAP by sonication. It was a convenient combination for separating and enriching cadmium, nickel, and lead in seawater. These metals are toxic, which have the WHO guideline value.¹ Cadmium is probably carcinogenic to humans (Group 2A) and nickel is carcinogenic to humans (Group 1).¹ The minimum measurable values of these metals are greater than the concentration of most environmental water, and the measurement of these metals is sometimes affected by the matrix, such as sea salt, and then separation and preconcentration are necessary. Different experimental parameters, such as the pH for chelate formation, the concentration of acid, and the sonication time for MVBE were optimized.

Experimental

Reagents and chemicals

Standard solutions (1000 mg/L) of Cd²⁺, Ni²⁺, and Pb²⁺ were purchased from Wako Pure Chemical, Japan. A tetramethylammonium hydroxide (TMAH) solution was diluted with a 25% TMAH solution (TAMAPURE AA grade, Tama Chemicals, Japan) to 12.5% with water. A 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol disodium salt (5-Br-PAPS)

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(0.4%) solution was dissolved 0.4 g of 5-Br-PAPS (Dojindo, Japan) in 100 mL of water. A phenolsulfonate (PS⁻, 0.5 mol/L) solution was dissolved 11.6 g of sodium *p*-hydroxybenzenesulfonate dihydrate (Kanto Chemical, Japan) in 100 mL of water, which was then purified by the addition of a 0.1% PAN {1-(2-pyridylazo)-2-naphthol} ethanol solution, followed by solid-phase extraction with Inertsep-C18 cartridges (GL Sciences, Japan). To prepare a benzethonium (Ben⁺, 0.1 mol/L) solution, 4.5 g of *N*-benzyl-*N,N*-dimethyl-2-[2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethoxy]ethan ammonium chloride (Kanto Chemical, Japan) was dissolved in 100 mL of water. An ammonium dihydrogen phosphate (0.5%) solution dissolved 0.5 g of ammonium dihydrogen phosphate (Wako, Japan) in 100 mL of nitric acid (2 mol/L). A mixed cellulose ester membrane filter (0.45 μ m pore) (Advantec, Japan) was used to filter the environmental water. All reagents were of analytical grade and water was obtained by using a Direct-Q 3UV (Millipore).

Apparatus

Measurements of trace metals were performed using a Hitachi (Japan) Model Z-5010 polarized Zeeman GF-AAS. Centrifugation was performed using a Kubota (Japan) centrifuge (Type 5420). A measurement of the pH was performed using a Horiba (Japan) Type D-53 pH/ion meter. For the entire sample preparation process, plastic centrifuge tubes (50 mL) were used. A Toshiba (Japan) home-use microwave oven (Model ER-250) with 500 W output power was used. ASU-10 CLEANER (AS-ONE, Japan) was used for sonication.

Procedure

An acidified sample solution (40 mL) containing 0.02 M nitric acid was taken into a centrifuge tube. As a chelating reagent, 0.4 mL of a 5-Br-PAPS solution and as both a component of the pH buffer and the organic anion 1.6 mL of a phenolsulfonate solution were added to the sample. The pH of the solution was adjusted to be about 9 using the TMAH solution. Then, 0.2 mL of the benzethonium solution as the organic cation was added to the operating solution. The solution was centrifuged at 3760g-force (4500 rpm) for 15 min. After centrifugation, the aqueous phase was discarded and a highly viscous IAP was remained at the bottom of the tube. The IAP was dried by microwave irradiation for 20 s for one tube. For back-extraction from the IAP, 75 μ L of 2 mol/L HNO₃ was added upon the dried IAP, and sonication was performed for 30 s. After the MVBE of the IAP, 25 μ L of an ammonium dihydrogen phosphate solution was added as a chemical modifier. The concentrate (10 μ L portion) was injected into a cuvette and the absorption was measured by GF-AAS at 228.8, 232, and 283.3 nm wavelengths for cadmium, nickel, and lead, respectively.

Environmental water samples were filtered through a membrane filter to remove any suspended solids. Seawater samples were spiked with a standard solution, which was done by following the procedure mentioned above. After discarding the aqueous phase, an additional cleanup step was performed to eliminate any interference from sea salt by washing the ion-associate by the supernatant liquid obtained by the centrifugation of a mixture of phenolsulfonate and benzethonium solutions, as reported in a previous study.¹⁷ The entire preconcentration procedure was performed in 40 min.

Fig. 1 Effect of the pH on the relative absorbance of trace metals. The relative absorbance was calculated from the absorbance found at different pH values divided by the highest absorbance found at pH ca. 9.

Results and Discussion

Chelate formation in IAP extraction/MVBE

Figure 1 shows the effect of the pH on chelate formation. In this study, a pH adjustment was performed by nitric acid, TMAH, and a phenolsulfonate solution ($pK_a = 8.56$).¹⁹ The optimum pH for the extraction of the metal/5-Br-PAPS complex in IAP of benzethonium and phenolsulfonate ion ranged over 8.5 – 9.5. At a high alkaline pH (≥ 10), the relative absorbances of the metals gradually decreased again because the acidity for MVBE became insufficient. IAP was not observed for a high alkaline pH (≥ 12). The effect of the pH on chelation formation is considered to be almost the same as in previous studies.^{17,18} However, at pH 10 or higher, the IAP extraction/MVBE technique was affected by the alkali component, it seems that the alkaline component attached to the IAP lowered the acidity of the nitric acid solution and interfered with the back extraction. For further experiments, pH 9.0 ± 0.5 was selected.

5-Br-PAPS was chosen as a chelating reagent, as reported concerning a previous study.^{17,18} The effect of the chelating reagent on the relative intensities of the metals was investigated by increasing the concentration of 0.4% 5-Br-PAPS from 0.1 to 0.8 mL (from 0.02 to 0.15 mmol/L). The chelating reagent solutions of 0.3 mL or less were not sufficient for complex formation with trace metals. Quantitative complex formation was obtained under 0.4 mL or more of a 5-Br-PAPS solution. Therefore, 0.4 mL (40 mL sample) was chosen for this experiment because the absorbance of trace metals was high, and the chelating reagent is expensive.

Micro-organic ion-associate phase (IAP) formation

In the present study, as an organic anion to form IAP, the phenolsulfonate anion was recommended because it served as a pH buffer component in previous studies.^{17,18} As an organic cation to form IAP, the effect of the benzethonium cation's concentration was studied ranged from 0.1 to 2 mmol/L. The amount of the IAP formed increased with increasing the concentration of the benzethonium cation. The excess amount of the IAP seems to require additional sonication time for stripping. The absorbance was highest when 0.5 mmol/L benzethonium cation was added. Then, the concentration of benzethonium 0.5 mmol/L was chosen.

The centrifugation conditions, such as the set-aside time and

Fig. 2 Effect for the micro-volume back-extraction (MVBE) of (A) different types of acids (2 mol/L) and (B) the concentration of nitric acid on the relative absorbance of trace metals. Cd: 12.5 ng/L, Ni: 200 ng/L, Pb: 7.5 ng/L; sonication time: 30 s. The relative absorbance was calculated from the found absorbance divided by the highest absorbance found when 2 mol/L of nitric acid was added for back-extraction.

the centrifugation rate and time, were optimized for this study. The set aside-time is the time interval between the addition of the benzethonium cation to the sample and the initiation of centrifugation for phase separation. The effect of the set-aside time, ranging over 0 – 60 min was examined. As the set-aside time before the centrifugation, ≥ 10 min, was chosen. The rate of centrifugation ranged over 3000 – 5000 rpm (1670 – 4640g-force), and centrifugation times of between 5 and 25 min were examined. As the centrifugation rate and time, 4500 rpm (3760g-force) and 15 min were chosen.

Micro-volume back-extraction (MVBE)

The purpose of this study was to design a method that can avoid the use of organic solvents for separating trace heavy metals. The study was conducted using three inorganic acids, *i.e.*, nitric acid, sulfuric acid, and hydrochloric acid (Fig. 2A). The absorbance was found to be the highest when nitric acid was used for MVBE. The effects of the nitric acid concentration on the absorbance were studied by increasing the concentration from 0.5 to 5 mol/L (Fig. 2B). The chosen concentration of nitric acid was 2 mol/L.

A study was conducted to optimize the sonication time after adding nitric acid for the back-extraction of metals from IAP. Sonication times ranging over 10 – 60 s were studied. It was observed that the sonication increased the absorbance compared with those without sonication, which proved that sonication facilitated back-extraction by accelerating the separation of trace metals from IAP. With sonication for 30 – 60 s, the absorbance-values of trace metals were almost the same. Therefore, 30 s was chosen.

Table 1 Analytical results for Cd of river-water samples (downstream)

River		Mean $\pm$ SD/ng L ⁻¹
Oyabe River	Jokoji Bridge ^a	9.9 $\pm$ 0.5
Sho River	Shinshogawa Bridge ^b	7.8 $\pm$ 0.6
Jinzu River	Hagiura Bridge ^c	25.5 $\pm$ 0.7
Joganji River	Imagawa Bridge ^d	6.2 $\pm$ 0.6

Oyabe River is the west, followed by Sho River, Jinzu River, and Joganji River at the east. These four rivers are located in Toyama Prefecture and flow into Toyama Bay in the Sea of Japan.

a. About 2.3 km from the river mouth.

b. About 1 km from the river mouth.

c. About 1.8 km from river mouth and *ca.* 59 km from the Kamioka mine, Hida city.

d. About 200 m from the river mouth.

$\pm$ the variation calculated from the standard deviation. No. of runs: 3.

Analytical figure of merit

The calibration graph of Cd, Ni, and Pb by the proposed method was linear with R^2 values of 0.998, 0.999, and 0.996 over the ranges of 5 – 25, 100 – 400, and 5 – 15 ng/L, respectively.

The IAP extraction method combined with a micro-volume back-extraction was a sensitive method because it could determine trace metals at lower concentrations (LOD: Cd 0.6 ng/L, 3.7 ng/L, and 0.8 ng/L level) than the recommended level of trace metals in water in global water quality guidelines¹ (3000 ng/L for Cd, 70000 ng/L for Ni, and 10000 ng/L for Pb).

Application to environmental water samples

Several decades ago, Itai-Itai disease occurred in the Jinzu River basin. Therefore, the Cd concentration in the Jinzu River was compared with the Cd concentration in other rivers (Table 1). The concentrations of Cd in all the river water in Table 1, including Jinzu River water, were much lower than the WHO guidelines.¹

The concentrations of Cd, Ni, and Pb in seawater were also determined and recovery tests of seawater spiked with Cd²⁺, Ni²⁺, and Pb²⁺ were performed. The results are given in Table 2. The concentrations of Cd, Ni, and Pb in seawater samples were also lower than the WHO guidelines.¹

Table 2 gives results of the recovery test in seawater, wherein the recoveries of Cd, Ni, and Pb in seawater were 86 – 104%. The achieved results proved the efficiency of the recommended method. For all cases, the experiment was performed three times, and about a 400-fold enrichment was obtained.

Conclusions

IAP extraction/micro-volume back-extraction (MVBE) using nitric acid was a sensitive, and highly enriched preconcentration and separation technique for trace heavy metals. It was successfully applied for separation, 400-fold enrichment, back-extraction, and GF-AAS determinations of Cd, Ni, and Pb in river water and seawater. An attractive feature of this technique is the requirement of a single vessel for the whole preconcentration process (*e.g.*, extraction and back-extraction), which thereby ensures a low possibility of contamination. The sample volume was downsized to 40 mL to minimize the required volume of the reagents, which reduced the volume of drainage after the experiments. Therefore, IAP extraction/MVBE using nitric acid is a simple, rapid, effective, and convenient

Table 2 Recovery tests for spiked Cd, Ni and Pb to seawater samples in Toyama Bay

Cd ^a				Ni ^b				Pb ^b			
Added/ ng L ⁻¹	Found/ng L ⁻¹ (Mean ± SD)	Recovery		Added/ ng L ⁻¹	Found/ng L ⁻¹ (Mean ± SD)	Recovery		Added/ ng L ⁻¹	Found/ng L ⁻¹ (Mean ± SD)	Recovery	
		ng L ⁻¹	%			ng L ⁻¹	%			ng L ⁻¹	%
0	4.4 ± 0.1	—	—	0	219 ± 2	—	—	0	15.1 ± 0.4	—	—
10	13.0 ± 0.8	8.6	86	200	414 ± 1	195	97	5	19.9 ± 0.3	4.8	96
20	24.5 ± 0.3	20.1	100	400	619 ± 1	400	100	10	25.3 ± 0.3	10.2	102
								15	30.8 ± 0.1	15.7	104

a. About 3 km from Shinminato shore, where is between the mouth of Sho River and the mouth of the Jinzu River. b. About 16 km from the mouth of the Jinzu River. No. of runs: 3.

method for the routine monitoring of trace heavy metals in environmental water.

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