On-site Determination of Trace Arsenic by Reflection-Absorption Colorimetry of Molybdenum Blue Collected on a Membrane Filter

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An on-site determination method for trace arsenic has been developed by collecting it as molybdenum blue (MB) in the presence of tetradecylmethylbenzylammonium chloride on a mixed cellulose ester membrane filter and by measuring reflection absorbance (RA) of MB on the filter using a laboratory-made palm-top size reflection-absorbance colorimeter with a red light-emitting diode. The value of RA was proportional to the amount of arsenic up to 0.5 μg with a detection limit of 0.01 μg. The proposed method was successfully applied to soil extract and hot-spring water samples.

Keywords Molybdenum blue, reflection, colorimeter, on-site analysis, arsenic, soil extract, hot-spring

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Introduction

Soluble inorganic arsenic is highly toxic for human health and its long-term ingestion from drinking water increases the risk of cancer. A provisional guideline value in WHO recommendations is 0.01 mg L⁻¹ for drinking water. The same value is adopted in the Japanese government’s notification. Concentrations of arsenic in fresh water are quite low, e.g., 0.004 mg L⁻¹ as an estimated mean for stream water in the world. Therefore, a simple and sensitive on-site analytical technique was required for the monitoring of environmental pollution of arsenic. The on-site technique is also useful to obtain proper analytical values by minimizing the influence of transfer time and/or storage of aqueous samples. As in the Gutzeit method, a visual detection limit (DL) of 0.01 mg L⁻¹ is performed by a commercial test kit based on the reduction of arsenic ions to arsine gas, the color change of mercuric bromide in a test strip, and the color comparison against a color calibration chart. The tristimulus colorimetric measurement was applied to the test paper prepared by the Gutzeit method. A determination limit of 0.001 mg L⁻¹ was reported for this technique. However, mercuric and tin(II) reagents used are toxic and therefore not recommended for routine analysis. The formation of micro particles of molybdosarenate with Ethyl Violet was used for the visual and spectrophotometric determination of arsenic in fresh water samples with DLs of 0.01 and 0.004 mg L⁻¹, respectively. A DL of 0.0075 mg L⁻¹ for As(V) was performed by a visual detection method using molybdenum-loaded chelating resin with β-hydroxypropyl-1-di(β-hydroxyethyl)amino moiety. But, this method was not quantitative. A portable arsenic analyzer with a DL of 0.0004 mg L⁻¹ was proposed for on-site water analysis. In this analyzer, AsH₃ was generated with NaBH₄ and chemiluminescence (CL) production of AsH₃ with O₃ was measured by a photomultiplier tube module. The analyzer does not require water or reagent solutions, but its O₃-generation part is heavy and the size of a CL cell unit is not small for on-site analysis. A laboratory-made portable instrument (6.5 kg) for anodic stripping voltammetry (ASV) is commercially available for the field determination of total As(III) and As(V) with a DL of 0.0005 mg L⁻¹. However, the interference from many species, e.g., surfactants, limits the application of ASV. We improved the molybdenum blue method and used it for on-site determination of soluble arsenic in soil samples. In this study, a new sensitive on-site method was developed based on the collection of molybdenum blue (MB) on a membrane filter and the measurement of reflection absorbance (RA) of MB. A laboratory-made palm-top size colorimeter using a red-green-blue light emitting diode (RGB LED) was modified and used for the measurement of RA. The proposed method was successfully applied to soil extract and hot-spring water samples.

Experimental

Reagents and samples

All chemicals were of analytical reagent grade. Ultrapure water with ≥18 MΩ cm was prepared by a Millipore Simpli Lab-UV system and used throughout. Working As(III) standard solutions were prepared by diluting Arsenic Standard Solution AS1000 (Kanto Chemical, 1.011 g L⁻¹ of As in 0.05% NaCl and HCl, pH 3) with water. A stock As(V) solution (1.00 g L⁻¹ of As in 0.1 M HCl) was prepared by dissolving As₂O₅ with 3 M HCl and diluting with water. Working As(V) standard solutions were prepared by diluting the stock solution with water. A molybdate solution (0.02 M, 2.5 w/v%) was prepared by

Notes

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dissolving $\text{(NH}_4\text{)}_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ with water. A Zephiramine (Zeph) solution (0.01 M) was prepared by dissolving benzyl-dimethyltetradecylammonium chloride dihydrate (Dojindo Laboratories) with water. L-Ascorbic acid (AA) solution (1.3 M, 20 w/v%) was prepared by dissolving the reagent (Wako Pure Chemical Industries) with water. A soil extract sample was prepared by extracting from 2.00 g of a soil sample with 20-mL water under ultrasonic irradiation at 46 kHz (18 W) for 30 min. The analytical sample was prepared by filtering the supernatant of extract with a mixed cellulose ester (MCE) membrane filter with a pore size of 0.45 $\mu$m. Water samples were analyzed after the filtration with the same type of membrane filter.

**Apparatus**

A laboratory-made portable reflection-absorption colorimeter ($120 \times 80 \times 55$ mm, 280 g including a battery) was based on the previously reported colorimeter and its schematics diagram is shown in Fig. 1. A cell holder was removed in order to measure reflection absorbance of MB on a filter. Instead of it, a bifurcated plastic-core optical fiber, couplers for the optical fiber to an LED or a photodiode, and the filter holder were added to the colorimeter. An RGB LED was used as a light source (three light emitters, 630, 530, and 470 nm). Two photodiodes (PDs) were used; one for the measurement of light intensity reflected by sample surface and the other for light source intensity. A bifurcated optical fiber was used to transfer the light from the LED to the sample (a filter) and bring the reflected light back to the PD. The absorbance value was displayed on a liquid-crystal display. Red light with a maximum emission wavelength of 630 nm and a spectral band width of 25 nm was used for the determination.

In order to confirm the reliability of the proposed method, the absorbance measurement at 840 nm was carried out with a double-beam spectrophotometer (Shimadzu, UV-160A or UV-2550) equipped with a 10-mm glass cell. Arsenic was also determined at 279.5 nm with an inductively-coupled plasma atomic emission spectrometer (Seiko Instruments, SPS 1500S or SPS 1700); 193.7 nm for As and 371.0 nm for Y as an internal standard.

A portable filtration system (Fig. 2) with a glass funnel, a glass filter holder, a plastic syringe tube and a hand manual suction pump was constructed and used for on-site filtration. The first two parts and a clamp for them are a commercial filtration kit for membrane filters with a diameter of 25 mm. An acrylic fixing frame was placed between the funnel and the filter holder in order to fix the filtration area (16 mm) in the fritted base and to perform homogeneous filtration. The filtration system was held with one hand. The filtration was carried out by ADVANTEC MCE membrane filters (Advantec, CA05A025A) with a pore size 0.45 $\mu$m and a filter diameter of 25 mm. Omnipore hydrophilic polytetrafluoroethylene (PTFE) membrane filters (Merk Millipore, JHWP) with the same pore size and diameter were also tested for the collection of molybdenum blue. Eppendorf micropipettes were used for on-site analysis. A portable vacuum stainless steel bottle with a wide opening and an immersion heater driven by a car adapter power supply was used for on-site heating. A commercial portable dryer was also used to dry membrane filters on-site.

**Analytical procedure**

The colorization of MB was carried out by the previous procedure for laboratory determination. An aliquot of the aqueous sample was placed into a 20-mL glass vial and diluted with water to adjust the volume (5.0 mL) of the final solution. Thereafter, 0.1 mL of 5 M sulfuric acid and 0.1 mL of 0.05 M potassium permanganate solution were added and mixed for about 0.5 min in order to oxidize As(III) to As(V). Purple color of permanganate ions was decolorized by adding 0.05 mL of 10 M urine solution and 0.05 mL of 1 M sodium nitrite solution. The excess of nitrite ions was decomposed completely by heating the solution at about 85°C for 0.5 min in the portable hot water bath. After cooling the solution in a water bath, 0.2 mL of 5 M sulfuric acid, 0.2 mL of 0.02 M molybdate solution and 0.5 mL of 1.3 M AA solution were added, and the
solution was heated at about 85°C for 15 min in order to form MB. After cooling, 0.015 mL of 0.01 M Zeph solution was added. The solution was mixed gently to minimize the formation of bubbles and then filtered with a membrane filter using the filtration system (Fig. 2). The filter was dried with the portable dryer and reflection absorbance \( (RA)_1 \) of MB was measured against a blank filter. When significant absorbance \( (RA)_2 \) was caused by phosphate ions, it was measured by adding 0.2 mL of 0.005 M of sodium thiosulfate solution before the addition of the molybdate solution. The amount of arsenic was determined by dividing the value of \( RA_1 - RA_2 \) by the slope of a calibration equation prepared previously in the laboratory.

### Results and Discussion

#### Collection of molybdenum blue on membrane filter

Anionic property of MB was utilized for its aggregation by the ion-pair formation with an organic matter and thereby the preconcentration of arsenic on a membrane filter.\(^\text{16}\) This property was also used for its preconcentration on a cationic exchanger.\(^\text{17}\) We preferred the former technique to develop simple on-site analysis by the measurement of reflection absorbance. As typical cationic and anionic surfactants, Zeph and sodium dodecylbenzenesulfonate (SDB) were tested for the collection of MB on a membrane filter. After the filtration of MB followed by the Analytical procedure, recoveries of MB on filters were obtained by absorbance measurements of filtrates. The recovery of MB containing 5 μg of arsenic was 9% at 0.2 mM of SDB. The solution became turbid at 0.3 mM of MB followed by the filters were obtained by absorbance measurements of filtrates. The recovery of MB containing

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**Preparation of membrane filter for measurement of reflection absorbance**

The MCE filter and the hydrophilic PTFE filter were tested to obtain homogeneous and strongly colored MB on the filter. The reproducibility of RA was evaluated by the measurement of the center of the filter and four concentric points on it. The colorization of MB on the MCE filter was visually clearer than that on the PTFE filter under dry and wet conditions. Therefore, the MCE filter was selected for the measurement of RA. In air-drying for 30 min, an average value of RA for the reagent blank was 0.044, which was five times lower than 0.218 for the wet filter. In the homogeneity test, a standard deviation of the four-points measurement for the dried filter was 0.002, which is five times lower than that for the wet filter. Thus, in the Analytical procedure, the measurement of RA was carried out for the MCE filter after drying the filter.

Air-drying was required for 1 h to reduce RA to a lower and constant value, e.g. 0.007 ± 0.002. The drying time is too long for on-site analysis. In this study, before the air-drying, the elimination of the wetness of the filter was tried by sandwiching with filter-paper, washing with petroleum ether and blowing hot air using a hair dryer. The total drying time was 40, 25 and 2 min, respectively. Therefore, a commercial portable hair dryer was used to minimize the drying time. The dryer gave low and reproducible values of RA (0.003 ± 0.001) for the reagent blank.

Homogeneity test of MB on the filter was carried out for filters with 0, 0.2, 0.4, 0.5 or 1 μg of As(III) or As(V). For all the filters, standard deviations of RA at the four concentric points were 0.002 or less. Within the error, values of RA at the central point agreed with averages of the four concentric points in respective amounts of arsenic. Therefore, unless otherwise noted, the measurement of RA was carried out at the center of the filter.

#### Determination of arsenic by the measurement of reflection absorbance

By the procedure in the Analytical procedure, the same calibration curve was obtained for As(III) and As(V) with a linearity up to 0.5 μg. In this linear range, arsenic was determined within an error of 0.02 μg. A value of DL corresponding to RA value three times the standard deviation of the reagent blank was 0.01 μg (n = 5). The value was 10 times smaller than that obtained by the enrichment by solvent extraction and colorimetry.\(^\text{18}\) The concentration of DL is 0.003 mg L\(^{-1}\), when 3-mL sample is analyzed by the proposed method. The concentration value of DL indicates that the method is applicable for monitoring water quality control.\(^\text{2,3}\)

#### Applications

River water collected in Kofu, Yamanashi, Japan, was analyzed by the proposed method as a representative of natural water. Hot spring water collected in Masutomi, Yamanashi, and soil extract were also analyzed as representative of water samples containing detectable amounts of arsenic and relatively large amounts of various constituents. Sample No. 2 in Table 1 was stored and analyzed as Sample No. 3. After sampling or extraction, solid particles in the sample were immediately eliminated by filtration through a membrane filter with a pore size of 0.45 μm. In the laboratory, the samples were stored at about 5°C and analyzed by the spectrophotometric molybdenum blue method\(^\text{14}\) and/or a inductively-coupled plasma atomic
emission spectrometry (ICP-AES) within one day after the sampling. The reflection-absorption colorimetry and the spectrophotometric molybdenum blue method required the correction of absorption caused by phosphate ions for all the samples analyzed. In all the samples, the maximum amount of phosphate ions in the sample taken was about 0.6 μg and it was corresponding to a RA value of 0.06. Table 1 gives the analytical results obtained by the above-mentioned three methods. The analytical result obtained by the proposed on-site method agreed with those by the other two methods within an error of 10%. Arsenic was not detected for the river water. In this sample, 97% of recovery was obtained with the addition of 0.2 μg of As(III). The storage test of a sample was carried out for Sample No. 2. Decreased analytical values were found for the stored sample (Sample No. 3), suggesting the usefulness of on-site analysis without the storage.

Table 1 Analytical results of arsenic in water samples

| No. | Sample            | Sample taken/mL | Concentration of arsenic/mg L⁻¹ |
|-----|-------------------|-----------------|---------------------------------|
|     |                   |                 | Reflection-absorption colorimetry | Spectrophotometry | ICP-AES |
| 1   | Arakawa river     | 3.5             | <0.003³ | <0.01³ | <0.03³ |
| 2   | Masutomi hot spring | 0.05           | 4.5, 4.6 | 4.8, 4.9 | 4.9 ± 0.2 |
| 3   | Masutomi hot spring | 0.02           | 1.8, 1.6 | — | 1.7 ± 0.2 |
| 4   | Soil extract      | 0.025           | 8.1, 7.3 | 7.9, 8.0 | 7.8 ± 0.3 |

a. In ICP-AES, the analytical value is indicated by average ± standard deviation in three determinations. Two analytical values are indicated for the other methods.
b. In the reflection-absorption colorimetry.
c. ICP-AES denotes inductively coupled plasma atomic emission spectrometry.
d. Values indicate detection limits for each analytical method. They are variable with the volume of sample taken.
e. Unfiltered Sample No. 2 was filtered after storage for 5 days, and then analyzed as Sample No. 3.

Conclusion

A sensitive reflection-absorption method was proposed for the on-site determination of trace arsenic in fresh-water samples. The collection of MB on the filter was studied for on-site enrichment and determination of arsenic. Arsenic, i.e., As(III) + As(V), can be determined within a DL of 0.003 μg L⁻¹ for a 3-mL sample. The proposed on-site method was applicable to river and hot spring water samples and soil extract.

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