STUDY ON WET DIGESTION METHOD FOR DETERMINATION OF TOTAL ARSENIC
IN MARINE ORGANISMS BY CONTINUOUS FLOW ARSINE GENERATION
AND ATOMIC ABSORPTION SPECTROMETRY USING SOME MODEL COMPOUNDS*

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A wet digestion method to determine total arsenic in marine organisms by continuous flow arsine generation and atomic absorption spectrometry was studied using some model compounds. Conditions for the generation of arsine were also studied for inorganic arsenite and arsenate, methylarsonic acid, dimethylarsinic acid, and phenylarsonic acid. The methylated arsenic species, phenylarsonic acid and arsenobetaine were completely decomposed to inorganic arsenate by the wet oxidation with nitric acid-perchloric acid-sulfuric acid system; an addition of nickel ion in the digestion process was not needed. Perchloric acid was essential to the complete decomposition of arsenobetaine when digestion was carried out on a hot plate.

INTRODUCTION

It has been shown that arsenic is contained at relatively high concentration in marine organisms in the forms of water-soluble and lipid-soluble compounds¹⁾. Recent studies on the identification of the chemical forms of arsenic in fresh-water and marine organisms have shown the presence of inorganic arsenite (As(III)) and arsenate (As(V)), methylarsonic acid (MAA), dimethylarsinic acid (DMAA)²⁾⁻⁵⁾, arsenobetaine (AB)⁶⁾⁻⁸⁾, arsenocholine moiety⁹⁾¹⁰⁾, 2-hydroxy-3-sulphopropyl-5-deoxy-5-(dimethylarsenosyl)-furanoside, and 2,3-dihydroxypropyl-5-deoxy-5-(dimethylarsenosyl)furanoside¹¹⁾. Identification and quantitative analysis of these arsenic compounds in marine organisms are important to estimate the toxicity and to investigate the metabolic pathway and accumulation of arsenic in sea ecosystems.

For the determination of total arsenic, dry ashing methods have often been used utilizing magnesium nitrate¹²⁾ or magnesium oxide - magnesium nitrate as ashing aid¹³⁾. Wet digestion has also been carried out utilizing nitric acid-perchloric acid-sulfuric acid system⁴⁾⁷⁾⁸⁾¹⁴⁾⁻¹⁶⁾. Hirayama et al.¹⁷⁾ have reported, however, that DMAA is lost during the wet digestion using the acid mixture and the loss of DMAA can be protected by adding nickel ion. They recommended the

* Wet digestion method for the determination of total arsenic in marine organisms (I).

addition of 500 mg of nickel ion for the decomposition of 1-5 g of hijiki (Hijikia fusiforme), konbu (Laminaria japonica) and shrimp, and arsenic in the resultant solutions were determined by graphite furnace atomic absorption spectrometry (GFAA). Arsine generation technique using sodium tetrahydroborate as a reductant is now widely used for the determination of arsenic¹⁸⁾⁻²³⁾ because it is rapid, sensitive, and semi- or full automated system using such as continuous flow system is easily applicable¹⁶⁾²⁰⁾⁻²³⁾. The addition of such a large amount of nickel in wet digestion process¹⁷⁾, however, results in severe interference in the arsine generation technique¹⁸⁾. Therefore present authors have studied to find a digestion method acceptable to the continuous flow arsine generation and atomic absorption spectrometry (CF-AGAA), using four organic arsenicals, i.e., MAA, DMAA, AB, and phenylarsonic acid (PhAA) as model compounds. Conditions for the generation of arsines derived from inorganic arsenic (As(III) and As(V)), MAA, DMAA, and PhAA by CF-AFAA were also studied.

EXPERIMENTAL

Reagents

Standard solution of As(III), 1 mg ml⁻¹: 0.660 g of As₂O₃ (Merck, 99.5% purity) was dissolved in 10 ml of 5 M NaOH and diluted to 500 ml with water.

Standard solutions of As(V), MAA, DMAA, and PhAA: Na₂HAsO₄·7H₂O (Wako Pure Chemical Co.), methylarsonic acid (99.5% purity), cacodylic acid (Nakarai Chemicals Co., 98% purity) and phenylarsonic acid were dissolved in water to be 1 mg ml⁻¹ as arsenic, respectively.

Standard solution of AB, 0.5 mg ml⁻¹ as arsenic: 0.066 g of arsenobetaine synthesized by the method of Edmonds et al.⁶⁾ was dissolved in water and diluted to 50 ml. The synthesized arsenobetaine contained: C, 30.80%; H, 6.65%. C₅H₁₁AsO₂·H₂O requires; C, 30.63%; H, 6.68%. The ¹H NMR spectrum (in D₂O) showed the same signal as literature⁶⁾.

Sodium tetrahydroborate solution, 1%: NaBH₄ (Aldrich Chemical Co.) was dissolved in 0.25% NaOH solution and filtrated with 1 μm glass microfibre filter.

All other reagents used were of analytical reagent grade.

Distilled-deionized water was used throughout.

Apparatus

A Varian-Thechtron model 175 type atomic absorption spectrophotometer equipped with an arsenic hollow-cathode lamp (Varian) was used. A modified Nippon Jarrel-ash HYD-1^{22,24)} was used for arsine generation; a Technicon proportioning pump module was employed to control the flow-rates of sample and reductant; a prepared sample solution and the reductant solution were mixed at the beginning of the reaction coil (16 mm i.d., 3 turns). Generated arsine was purged by nitrogen gas which was introduced into an open-ended quartz tube atomizer (14mm i.d., 114 mm long) mounted on the air-acetylene burner head by metal clips. The absorption signals were recorded on a NEVA 12 MS strip-chart recorder.

The instrumental operating conditions were as follows: wavelength, 193.7 nm; spectral slit width, 1.0 nm; arsenic lamp current, 7 mA; damping, "slow"; flow-rate of air and acetylene gas for burner were 12.8 l min⁻¹ and 1.7 l min⁻¹, respectively; nitrogen purge gas flow-rate, 0.5 l min⁻¹. The flow-rate of sodium tetrahydro-

borate solution and that of sample solution were both 3.9 ml min^{-1} in all instances.
 Procedure

Decomposition of organic arsenic compounds: The standard arsenic solutions ($20 \mu\text{g}$ as arsenic, 4 ml of $5 \mu\text{g ml}^{-1}$ solution) and digestion reagents were pipetted in a 50-ml conical beaker and covered with a watch glass, and heated on a hot plate (Yamato HK-21, slightly in excess of 300°C is attainable as a maximum temperature)—details are described in the captions of Table 1.

Procedure for CF-AGAA: After instrumental setting, the reductant solution and a blank solution were pumped until the recorder indicates stable base line. A sample solution was passed for 70 s, then a blank solution again for 120 s.

RESULTS AND DISCUSSION

Continuous flow arsine generation and atomic absorption

Conditions for the generation of arsine derived from As(III), As(V), MAA, DMAA, and PhAA were studied at first. Figure 1 shows the effect of the concentration of hydrochloric acid on the generation of arsines. The sensitivity for As(V) is lower than that for As(III) in the range of 0.2–4.5 N hydrochloric acid, but almost same at 4.5 to 8 N. When the sample solutions are prepared with 2% potassium iodide and 3% hydroxylamine as reported previously²³, the sensitivity for As(V) in 0.8–4.5 N hydrochloric acid is enhanced to the level that for As(III). The sensitivities for MAA and DMAA have maxima in 1–2 N and 0.5 N hydrochloric acid, respectively; they correspond to 86% and 84% of that for As(III), respectively. Although the sensitivities for MAA and DMAA were both decreased with increasing the acidity

Fig. 1 Effect of acidity on the generation of arsines

Arsenic concentration: 20 ppb
 (o) HCl only,
 (●) HCl + 2% KI + 3% NH₂OH·HCl

to 8 N as reported by Hinners using batch method²⁵⁾, they were increased by preparing the solution in 2% potassium iodide and 3% hydroxylamine in the 5-8 N hydrochloric acid for both compounds. The sensitivity for PhAA is about 10% of that for As(III), and this was not improved by adding potassium iodide and hydroxylamine solutions.

No absorbance was observed for 0.5 ppm AB in all acidity range examined.

Wet digestion of organic arsenic compounds

The four organic arsenic compounds were digested with various acids-oxidizing reagent systems. One of the methods to evaluate the decomposition percent of methylated arsenic compounds to As(V) is the application of the differential determination of arsines after separation of them according to their boiling points²⁾³⁾⁵⁾. More simple and rapid method was used in this paper utilizing the CF-AGAA. The decomposition percent of the arsenic species to As(V) was evaluated by comparing the absorbance measured with 8 N hydrochloric acid to that of standard As(V) solution. Measurement in the acidity was settled because it can be minimize the error derived from undecomposed MAA and DMAA as can be seen from Fig. 1.

Table 1 shows the decomposition percent of organic arsenicals to As(V). For the sample which showed incomplete decomposition, the loss of arsenic during the decomposition process was checked by re-measuring in 1.0 N and 0.5 N hydrochloric acid for the digested solution of MAA and DMAA, respectively, or by repeating the digestion with $\text{HClO}_4\text{-H}_2\text{SO}_4$ system for AB and PhAA. No loss of arsenic during digestion was confirmed by these methods for all instances summarized in Table 1. As shown in Table 1, nitric acid and hydrogen peroxide are not effective for complete decompo-

Table 1. Decomposition percent of organic arsenic compounds to As(V)

Digestion system ^{a)}	MAA	DMAA	AB	PhAA
1 HNO_3	0, 0	0, 0, 0	0, 0, 15	20, 40
2 $\text{HNO}_3\text{-H}_2\text{O}_2$	50, 50 53	0, 10	0, 0, 12	33, 45
3 $\text{HNO}_3\text{-HClO}_4$	100, 100 100	95, 97, 100 100, 100, 100	100, 100	100, 100
4 $\text{HNO}_3\text{-H}_2\text{SO}_4$	100, 100 100	96, 98, 100	50, 60, 75 85	100, 100
5 $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$	100, 100, 100	96, 98, 100, 100, 100, 100	96, 98, 100, 100, 100	100, 100
6 $\text{HNO}_3\text{-(NH}_4)_2\text{S}_2\text{O}_8$	98, 98 100, 100	90, 96, 100 100	10, 15, 27	40, 50, 70
7 $\text{NaOH-(NH}_4)_2\text{S}_2\text{O}_8$	95, 100	100, 100, 100	95, 100, 100	93, 100

a) Amounts of the reagents used were as follows: HNO_3 (63%), 10 ml; H_2O_2 (30%W/V), 5 ml; HClO_4 (60%), 5 ml; H_2SO_4 (conc.), 3 ml; $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (2M), 3 ml; NaOH (5M), 5 ml. Heating was continued until nearly dryness (170-190°C for systems 1, 2, 6 and 7; 230-250°C for system 3), or until the volume became below 1 ml (>300°C) for systems 4 and 5.

sition. The systems containing perchloric acid are all effective for the purpose. The poor reproducibility was observed for the decomposition of AB by nitric acid - sulfuric acid system. However, AB was quantitatively decomposed to As(V) in the same system by Kjeldahl method (decomposition, 100%; $n=8$, $RSD=2\%$; heating was continued for 3 hours after fumes of sulfur trioxide appeared). Peroxodisulfate is effective in alkaline solution for the four arsenic species, but not in acidic solution for AB and PhAA.

As described above, nitric acid - perchloric acid - sulfuric acid system is satisfactory, and an addition of nickel ion in the decomposition process is not needed. The same results were obtained for DMAA when the watch glass on the beaker was removed or the heating was carried out on a sand bath.

The differences of the results between the effectiveness of nickel ion¹⁷⁾ and unnecessary of it during wet digestion process will be discussed in a subsequent paper in detail.

Effect of diverse ions on the determination of total arsenic

As described above, recommended condition for the determination of total arsenic is 1-4.5 N HCl medium with 2% KI and 3% $NH_2OH \cdot HCl$ after digestion was carried out in the acid mixture; the calibration curve for standard solution of As(V) is linear up to 40 ppb. Relative standard deviation calculated from ten determinations of 20 ppb As(V) solution was 2.1%. In the determination of As(V) (20 ppb), coexistence of following ions is acceptable up to 1000 ppm of BO_3^{3-} , SO_4^{2-} , ClO_4^- , IO_3^- , $C_2O_4^{2-}$, NO_3^- , CH_3COO^- , Cl^- , Br^- , and Al(III); 100 ppm of SiO_3^{2-} , PO_4^{3-} , Ru(III), Zn(II), Mn(II), Mn(VII), Cr(III), Cr(VI), Mo(IV), and Fe(III); 50 ppm of Cd(II) and Se(VI); 10 ppm of Bi(III), Ge(IV), Hg(II), Cu(II), Ag(I), and Co(II); 5 ppm of Sn(II); and 2 ppm of Ni(II). Interference caused by nickel ion in the recommended condition is less than that in HCl matrix (Fig. 2), and it is more eliminated by using EDTA or o-phenanthroline instead of 2% KI and 3% $NH_2OH \cdot HCl$: As shown in Fig. 2, 0.1% o-phenanthroline enabled the coexistence of 100 ppm Ni(II) ion.

Fig. 2 Interference of nickel ion on the arsine generation and its elimination

As(V) 20 ppb in 1 N HCl,
 (O) HCl only,
 (▲) 2% KI + 3% $NH_2OH \cdot HCl$,
 (Δ) 0.01 M EDTA·2Na,
 (●) 0.1% o-phenanthroline

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