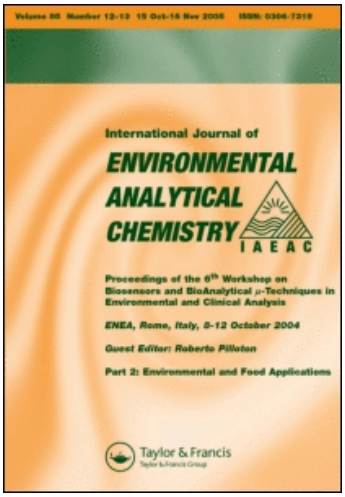

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Picogram Determination of Methylmercury in Seawater by Gold Amalgamation and Atomic Absorption Spectrophotometry

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An analytical procedure for the determination of total mercury (T-Hg) and methylmercury (CH_3Hg) in seawater was described. CH_3Hg was extracted with benzene and concentrated by a succession of three partitions between benzene and a cysteine solution. From the last cysteine extract, CH_3Hg was transfered into a dithizone chloroform solution. CH_3Hg-dithizonate was determined by atomic absorption spectrophotometry (AAS). T-Hg was also determined by AAS after wet combustion of the sample with sulfuric acid and potassium permanganate. The limit of detection was 0.005 ng for CH_3Hg, when 3.6 liter of seawater was treated, and the coefficients of variation for CH_3Hg 10 ng was about 1%. Seawater from the coast of the Kinki-area, the middle part of the mainland of Japan, seemed to have a T-Hg content of about 10 ng l^{-1} of which nearly 1% was suspected to be the content of CH_3Hg.

KEY WORDS: Seawater, methylmercury, total mercury, preconcentration, enhancement in sensitivity.

INTRODUCTION

Fish in water containing methylmercury (CH_3Hg) at a low level of ppt concentration[1,2] can accumulate CH_3Hg to a concentration at the ppm level. The concentration factor of CH_3Hg for fish[3] ranges 10^3 to 10^5. The total mercury content (T-Hg)[4,5] in seawater has been reported as around 10 ng l^{-1}. However, the proportion of

CH_3Hg to the T-Hg in seawater has remained uncertain having been reported about 10%[1], 30%[6] and some others. This seems to be due to the insufficient sensitivity of recent analytical techniques to determine extremely low concentration of CH_3Hg precisely.

We, therefore, have studied on the preconcentration of CH_3Hg by a series of partitions. In order to obtain the desired sensitivity of CH_3Hg by the cold vapor atomic absorption spectrophotometry (AAS), we have made investigations on optimization of the spectrophotometry. The proportion of CH_3Hg to the T-Hg in three onshore seawater samples will be examined.

MATERIALS AND METHODS

Reagents

Chemicals in a certified reagent grade were used through our studies.

Working standard solutions

A stock solution of mercuric chloride ($1000\,\mu g\,l^{-1}$) and a methylmecuric chloride stock solution ($1000\,\mu g\,l^{-1}$) were used for all standards. Both solutions were used for AAS measurements after appropriate dilution and calibration of which procedure in Procedure section.

Dithizone solution

Dithizone (100 mg) was dissolved into 50 ml of chloroform, and extracted three times with 30 ml portions of 0.3% ammonia water. The water layer was made slightly acidic with an 18% hydrochloric acid solution, then extracted twice with 100 ml portions of chloroform. The chloroform extracts were combined and diluted to 1 liter with chloroform.

Cysteine solution

L-Cysteine hydrochloride (1.0 g), sodium acetate (0.8 g) and anhydrous sodium sulfate (12.5 g) were dissolved in 100 ml water. A fresh solution was made for each set of analysis.

Removal of mercury from reagents and utensils

Mercury in hydrochloric acid and sulfuric acid was eliminated by bubbling with air of which mercury had been removed by filtering with gold sand after addition of 5 drops of a 5% tin(II) chloride solution to the acids. Sodium chloride, calcium oxide and sodium sulfate (anhydrous) were heated at 600°C for 6 h. Glassware which withstood heat was heated at 500°C for 1 h. Quartz boats for AAS were heated at 800°C for 1 h.

Methods of sampling and preserving seawater samples

Surface water samples were collected within Pyrex bottles (volume of 2 liter each) with ground-in glass stoppers, which were previously heat-treated and rinsed well with seawater at the sampling points 100 m away from the seashore. Immediately after the sampling, samples for T-Hg determination were made in 1 N acidic solution with sulfuric acid, and the samples for CH_3Hg determination were made in 1 N solution of hydrochloric acid with NaCl-saturation.

Samples of seawater were collected in Hyogo Prefecture, the middle part of the mainland of Japan, and the places were shown in Figure 1. A sample of groundwater was collected in the New Kobe Tunnel, Hyogo Prefecture, and a sample of riverwater which seemed to have suffered no artificial contamination, was collected from the Kurobe River in Toyama Prefecture. A table of samples are shown in Table I.

Apparatus

An atomic absorption spectrophotometer (Nihon Jarrell-Ash, Model 8200), mercury hollow cathode lamps (Hitachi, Hamamatsu-Television and Westinghouse), and a manganese lamp (Hamamatsu-Television) were used. As an analytical line for mercury, 2537 Å was used. Simultaneous correction of the background was performed using a manganese line of 2527 Å.

Sample decomposition part (Figure 2A)

A 50-mm section of cupric oxide on activated alumina, and a 5-mm section of a calcium oxide granule were placed in a quartz tube

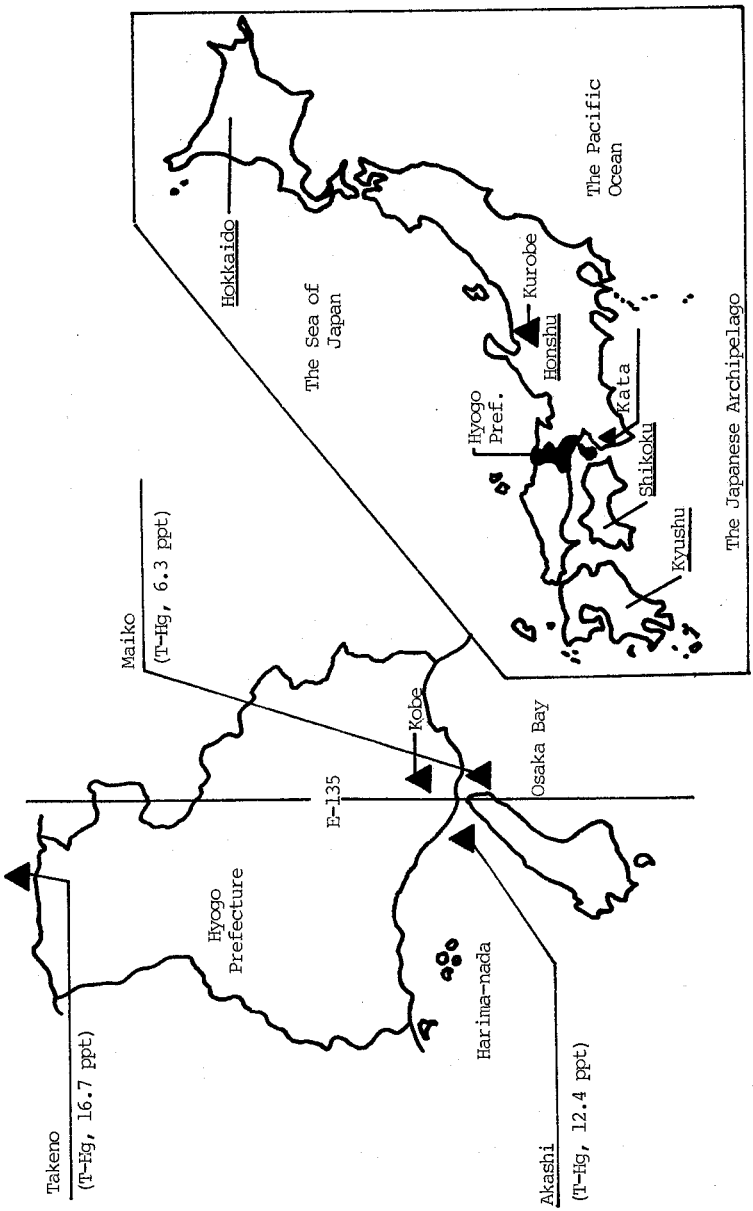

FIGURE 1 Places of sample collection.

TABLE I

Samples

Sample	Place collected	At sampling	
		Temp. (°C)	pH
Seawater A	Japan Inland Sea	15.5	7.9
Seawater B	Japan Inland Sea	14.5	8.0
Seawater C	Japan Sea	16.5	8.3
Groundwater	New Kobe Tunnel, Kobe, Japan	—	—
Riverwater	Kurobe river Toyama Pref. Japan	—	—

(20 mm i.d., 300 mm in length). A cupric oxide granule was prepared by adding activated alumina (Merck, 300 mesh, 5 g) into fused $CuNO_3 \cdot 3H_2O$ (3.8 g) and calcination with mixing. Air (Figure 2a) flow into this system was demercuried by gold sand (Figure 2b), which was prepared by quartz sand (fine granular, washed and calcinated, 50–80 mesh, 5 g) with gold (1 g) in aqua regia. The water and acid were evaporated from the mixture under heating and mixing to produce a metalic coating with gold surface.

Interferant removing part (Figure 2B)

Sulfides in the outbroken gas from the sample decomposition part (Figure 2A) were removed by CuO on alumina (Figure 2f), and halides with calcium oxide (Figure 2g). Acidic substances and oily materials in the gas were eliminated with an alkaline solution (Figure 2i), and the gas was dried at the U-shaped tube (Figure 2h), then elemental Hg was allowed to absorb on gold sand (Figure 2k; at room temperature) in an analytical column which consists of a 20 mm section of gold sand in a quartz tube (5×200 mm). Hg^{2+} and

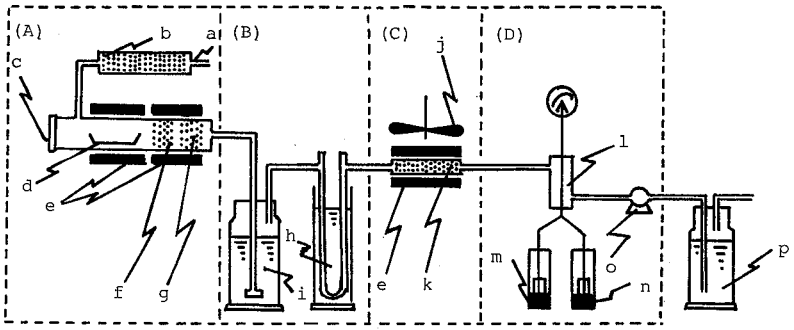

FIGURE 2 Schematic diagram of apparatus. (A) Sample decomposition part. (B) Interferant removing part. (C) Mercury collection part. (D) Mercury detection part. a—air; b—gold quartz; c—sample inlet; d—quartz boat; e—tubular oven; f—CuO; g—CaO; h—cold bath; i—1 N NaOH solution (5% $SnCl_2$); j—fan; k—analytical column (gold quartz); l—optical cell; m—hollow cathode lamp (Hg, 2537 Å); n—hollow cathode lamp (Mn, 2527 Å); o—accumulator pump; p—mercury absorption solution ($KMnO_4$-H_2SO_4).

HgO in water droplets accompanied by the gas were reduced to Hg in the alkaline solution containing stannous chloride (Figure 2i).

Mercury collection part (Figure 2C)

The elementary Hg absorbed on the analytical column (Figure 2k) was then led into the optical cell (Figure 2l) by heating the column at 500°C.

Mercury detection part (Figure 2D)

Hg vapor from the analytical column was led to the optical cell where mercury concentration was measured by AAS. As an optical gas cell, efficacy of cells X (13×135 mm, 17.9 cm^3), Y (18×200 mm, 50.9 cm^3) and Z (25×100 mm, 49.1 cm^3) was tested. After investigation (see Results section), the cell X was chosen for the purpose.

Procedures

Total mercury in water sample: A water sample (200–500 ml) was made in 1 N solution with sulfuric acid, and heated in a boiling

water bath for 1 h after addition of a 2% potassium permanganate solution until it turned violet. After cooling, a 5% hydroxylamine hydrochloride solution was added until the color of the solution just faded, then extracted with 5 ml of a 0.01% dithizone chloroform solution. One milliliter of the extract was taken in a quartz boat, and chloroform was evaporated in a stream of air at 40°C. The dithizonate residue in the boat was placed in the sample decomposition part (Figure 2d), heated at 900°C with aspirating by a pump (Figure 2o). The analytical column (Figure 2k) which absorbed the elemental Hg from the sample was heated at 500°C to generate Hg vapor. The vapor went into a gas cell (Figure 2l) for determination of mercury by AAS.

Methylmercury in seawater: A water sample (3,600 ml) was made in 1 N solution with hydrochloric acid and extracted four times with 500 ml portions of benzene after saturation with sodium chloride and addition of 20 ml of a 10% cuprous chloride solution. The benzene extract was combined and washed three times with 100 ml portions of a 20% sodium chloride solution, then extracted with 100, 60 and 50 ml portions of a 1% cysteine acetate solution, successively.

The cysteine extract was combined and added 100 ml of a 6 N hydrochloric acid solution, then extracted with 100, 100, 60 and 50 ml portions of benzene, successively. The partition between benzene and cysteine solutions was further repeated twice during which one volume of the former extract was extracted with one tenth volume of the following solvent. To the last extract with a cysteine solution, 5 ml of a 6 N hydrochloric acid solution was added and extracted with 4 ml of a dithizone chloroform solution. The dithizone extract (3 ml) was evaporated in a boat to analyze by AAS. The procedures for a typical seawater sample are illustrating schematically as shown in the Scheme (Table II).

RESULTS

1. Influence of the optical cell volume and the flow rate of carrier gas for AAS on the sensitivity of mercury detection

After 10 ng of elemental Hg was subjected to absorb on the analytical column (Figure 2k) at room temperature, the column was

TABLE II

Scheme for the determination of methylmercury in seawater sample

Seawater 3600 ml
- NaCl 360 g
- 12 N HCl 720 ml
- 10% CuCl in 4 N HCl 20 ml
- Extract with 540 ml portions of benzene four times with shaking for 60 min each.

Benzene extract
- Wash with 100 ml portions of 20% NaCl in H_2O solution three times.

Benzene extract 2100 ml
- Extract with 100, 60 and 50 ml portions of 1% cysteine solution with shaking for 15 min each.

Cysteine extract 205 ml
- 6 N HCl 100 ml
- Extract with 100, 100, 60 and 50 ml portions of benzene with shaking for 40 min each.

Benzene extract
- Wash with 20 ml portions of 20% NaCl solution three times.

Benzene extract*
- Extract with 15, 10 and 10 ml of 1% cysteine soln. with shaking for 30 min each.

Benzene extract* 300 ml

Cysteine extract 30 ml
- 6 N HCl 15 ml
- Extract with 15 ml portions of benzene three times with shaking for 30 min each.

Benzene extract
- Wash with 5 ml portions of 20% NaCl solution three times.

Benzene extract 40 ml
- Extract with 6 and 5 ml portions of cysteine solution with shaking for 20 min each.

Cysteine extract 10 ml
- 6 N HCl 5 ml
- Extract with dithizone-$CHCl_3$ 4 ml

Dithizone extract 3 ml
- Air-dry in a boat at 40°C

Hg-dithizonate

▼

AAS

heated at 300°C to generate Hg vapor. The vapor went into one of the gas cells (Figure 2l), e.g., the cell X, Y or Z. Figure 3 shows effect of carrier gas flow rate on the peak height responses (mm/10 ng) using one of the cell X, Y or Z. The highest response for 10 ng Hg by every cell was obtained at the lowest flow rate (0.25 liter min^{-1})

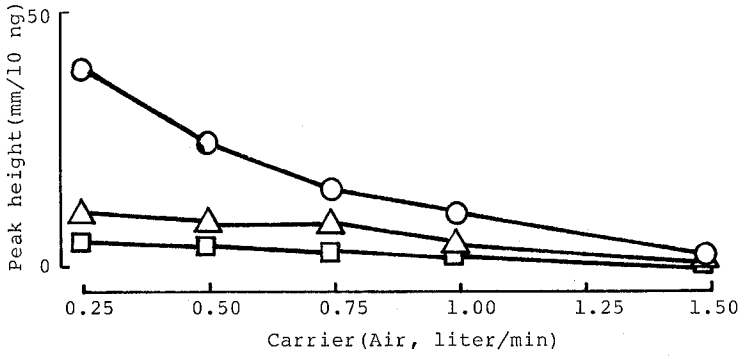

FIGURE 3 Effect of carrier gas flow rates on Hg-release at 300°C. Optical cell X ○——○; 13 × 135 mm (17.9 cm^3): Y △——△; 18 × 200 mm (50.9 cm^3): Z □——□; 25 × 100 mm (49.1 cm^3). Applied sample; Hg 10 ng. Instrument; Nihon Jarrell-Ash AA-8200.

within the examined range of the rates. The much lower flow rates gave broad peak responses inconvenient for Hg-analysis.

The volume of the cell X is roughly one third of the cells Y and Z, and 4-fold high responses were obtained by use of the cell X than those by the cells Y and Z in every flow rate under heating at 300°C. The cells Y and Z have nearly the same volume, and we have compared the responses by use of those two. In a given cell volume, the longer in length and the smaller in diameter, higher responses have been obtained as shown in Figure 3.

2. Relationship between the heating temperature to release mercury vapor from the absorbent and the sensitivity of mercury detection

The cell X was, therefore, chosen for its efficacy of mercury detection, and we investigated the effect of heating temperature on amalgam decomposition in the analytical column (Figure 2k) on the responses using the cell X in the flow rate of 0.25 l.min^{-1}. The results obtained were shown in Figure 4.

Compared with the responses at 300°C, it gave double-high responses when the column was heated at 500°C. In what follows, accordingly, we determined Hg concentration under the condition of amalgam decomposition at 500°C in carrier flow rate of 0.25 l.min^{-1} by use of the cell X.

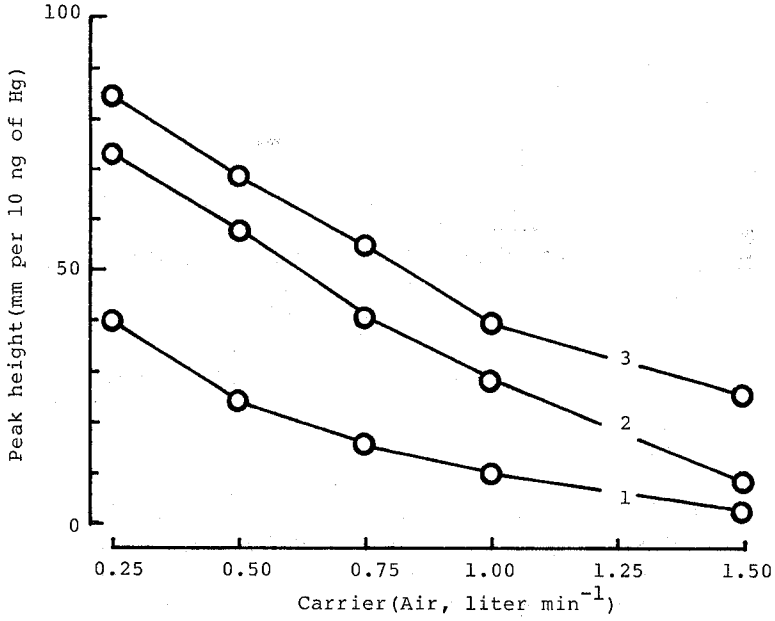

FIGURE 4 Effect of carrier gas flow rates on Hg-release at various temperatures with respect to the optical cell X. Applied amount of Hg was 10 ng each. Remarks: 1, Hg release at 300°C; 2, at 400°C; 3, at 500°C.

3. Preconcentration of methylmercury

As shown in Scheme 1, cleanup and preconcentration of CH_3Hg by the partition recurred three times between benzene and a cysteine solution cause 1000-fold concentrated CH_3Hg solutions theoretically compared with the initial sample.

It is necessary to investigate the effectiveness of the CH_3Hg partition between the last cysteine extract and a dithizone solution as well as the effectiveness of elimination of inorganic mercury by the partition. The partition was applied to 10 ml of cysteine solutions containing CH_3Hg 5 ng, containing CH_3Hg (5 ng) and Hg^{2+} (20 ng), and the solution containing only Hg^{2+} 20 ng under the acidity of hydrochloric acid from 0.5 to 3.0 N. Hg^{2+} was not detected as CH_3Hg under the acidity from 0.5 to 1.5 N, but detected a little as CH_3Hg signal between the acidity 2.0 and 3.0 N (Fig. 5). Between the acidity 1.0 and 3.0 N, CH_3Hg was quantiatively recovered.

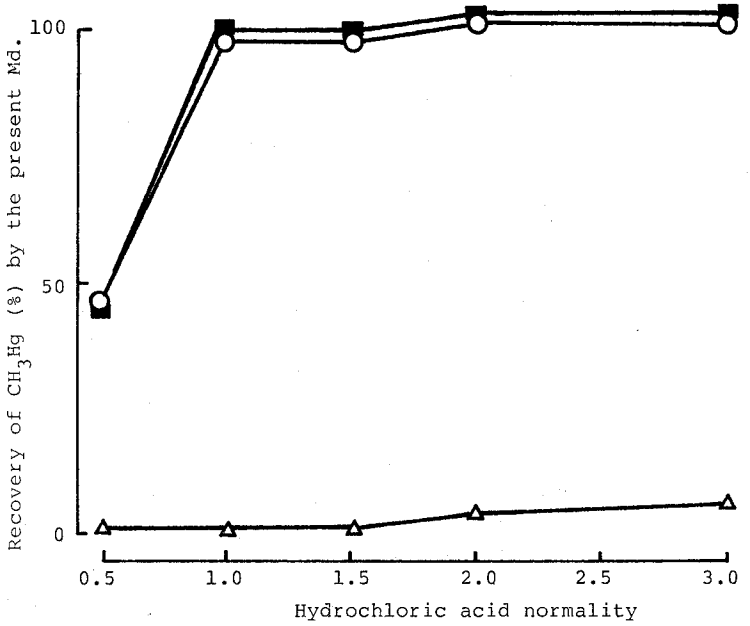

FIGURE 5 Extraction of methylmercury with dithizone-$CHCl_3$ from cysteine solution containing Hg^{2+} at various normalities. ○——○ Recovery from cysteine solution fortified CH_3Hg 5 ng. ■——■ From the solution fortified CH_3Hg 5 ng and Hg^{2+} 20 ng. △——△ From the solution fortified Hg^{2+} 20 ng.

On the basis of these data, subsequent experiments were carried out by dithizone extraction from the last cysteine solution extract adjusted to the acidity of 2.0 N. From the results mentioned above, total procedure for CH_3Hg determination was built up and was summarized in Scheme 1.

4. Application of the proposed method to some water samples

The total procedure was applied to artificial seawater, and the results of the recovery test were shown in Table III. Fortified Hg^{2+} was scarcely recovered as CH_3Hg, and the responses of fortified CH_3Hg only were observed. Taking into account the S/N ratio, the detection limit of CH_3Hg is presumed to be 5 pgl^{-1}. As with the artificial seawater containing CH_3Hg 9.7 ng, and the water containing CH_3Hg

TABLE III

Recovery of methylmercury

Sample	Fortified Hg^{2+} (ng)	Fortified CH_3Hg (ng)	Found as CH_3Hg (ng)	Recovery (%)
3.6% NaCl in	1000	—	0.01	—
distilled water	—	9.70	9.20	94.8
(3.6 liter)	1000	9.70	9.30	96.9

9.7 ng and Hg^{2+} 1000 ng, fortified CH_3Hg was quantiatively recovered. Accordingly, the procedure seemed to be applicable to seawater samples.

CH_3Hg content in some seawater was determined with/without CH_3Hg and Hg^{2+} fortification, and the results obtained were listed in Table IV. In the seawater samples of A, B and C, T-Hg approx. 10 ng l^{-1} and about 0.1 ng l^{-1} of CH_3Hg were found, nearly 1% of T-Hg corresponded to that of CH_3Hg. To a groundwater and a riverwater sample seemingly suffering from no artificial contamination, the procedure was also applied. Neither of the samples has been found to be contaminated with CH_3Hg.

DISCUSSION

In order to achieve the quantiative detection of CH_3Hg at a ppt level in seawater, we have studied on obtaining a ten thousand-fold concentrated CH_3Hg sample solution as well as enhancement in the sensitivity of AAS.

The highest response of mercury by AAS in our studies has been obtained by rapid decomposition of amalgam to release mercury vapor by heating the analtyical column at a high temperature, by passing the carrier gas in a low flow rate to introduce elemental mercury into an optical cell, and by use of an optical cell which is small in volume and short in diameter.

Extraction of CH_3Hg from a large quantity of seawater (80 liter) and preconcentration with XAD-2 resin had been tried[1] with the detection limit of 0.1 ng l^{-1}, and in another report,[7] the limit was 5 ng l^{-1}.

TABLE IV

Results of application of the proposed method to some seawater samples

Seawater sample	T-Hg ($ng\,l^{-1}$)	① CH_3Hg ($ng\,l^{-1}$)	CH_3Hg/T-Hg (%)	Fortified ($ng\,l^{-1}$)		② Found as CH_3Hg ($ng\,l^{-1}$)	Recovery (%)	②/① (%)
				Hg^{2+}	CH_3Hg			
A	12.40	0.156	1.26	1000	—	0.168	—	107.7
				—	9.70	8.973	90.0	—
B	6.30	0.040	0.63	1000	—	0.050	—	125.0
				—	9.70	8.644	88.7	—
C	16.70	0.100	0.60	1000	—	0.100	—	100.0
Groundwater	1.30	<0.005	—	—	—	—	—	—
Riverwater	0.70	<0.005	—	—	—	—	—	—

Samples A and B; coastal seawater of Japan Inland Sea.
Sample C; coastal seawater of Japan Sea.
Groundwater; New Kobe Tunnel, Kobe, Japan.
Riverwater, Kurobe River, Toyama Prefecture, Japan.

Selective preconcentration of CH_3Hg from a 3.6 liter of seawater sample was performed by the benzene-cysteine solution partitions repeated three times. Efficacy of CH_3Hg extraction and removal of inorganic mercury by the partition have already been proven in the Official Method.[8] After digestion of CH_3Hg in the last cysteine solution, mercury was reduced with stannous chloride to evolve mercury vapor for AAS. Determination of 0.1 ng level of CH_3Hg could hardly be performed by the high background peaks equivalent to around 5 ng of mercury through the reagents used at this stage.

So as to obtain much concentrated CH_3Hg sample solutions for AAS with low background, CH_3Hg was completely transfered into dithizone chloroform solution from the last cysteine solution extract. Such a treatment makes it possible to apply most of the extracted CH_3Hg into the sample boat for AAS. Accordingly, 10-fold concentrated CH_3Hg is brought about by use of the cysteine-dithizone partition. This stage accompanied by the former partition step enables to detect total absolute quantity of 0.01 ng of CH_3Hg in seawater sample of 3.6 liter with detection limit of 0.005 ng under the revised operating conditions of AAS. To explain this stage in detail; CH_3Hg in acidic (2 N HCl solution) cysteine solution passes into dithizone chloroform layer to form CH_3Hg dithizonate, but mercury cysteinate is stable and is retained in water layer as it is (Figure 5).

Even by the repeated partition in our experiments, 3.6% of inorganic mercury leaked through from the cysteine solution into the benzene layer for each partition, meanwhile, the succeeding dithizone extraction step should eliminate almost all the inorganic mercury (Figure 5). As shown in Table III the migration of inorganic mercury into the final sample solution for AAS was negligible or nearly the detection limit (0.005 ng) with quantiative recoveries of fortified CH_3Hg.

Extraction of CH_3Hg with benzene from sample water is disturbed in the presence of sulfides, thiocyanates and some other species.[9] This disturbance is eliminated by use of cuprous chloride in acidic solution following the JIS K 0102.[8] In association with these factors, some part of CH_3Hg in seawater is held in suspended substances (SS, mostly phytoplankton).[1]

In order to overcome those difficulties, Matsunga *et al.*[5] let the acidic seawater sample stand for two weeks to breakdown the SS. We also follow the procedure adjusting the acidity of the sample

water to 1 N with hydrochloric acid as well as adding cuprous chloride and sodium chloride.

In seawater samples A, B and C, T-Hg about 10 ng l^{-1} was found (Table IV). About 90% of the fortified CH_3Hg (9.7 ng l^{-1}) to the sample A and B have been recovered by the procedure, but they were rather lower values.

From the recoveries, we estimate that a small part of CH_3Hg in the samples cannot be extracted due to the complex formation of CH_3Hg with some sulfides or SS even by CuCl-NaCl-HCl treatment. Fortification of Hg^{2+} (1000 ng l^{-1}) to the sample A and B resulted in much higher (110–120%) CH_3Hg values than the initial determination. On the other hand, the measured CH_3Hg value on the sample C with/without Hg^{2+} fortification almost agreed. As shown in Table III it is possible that a little of the fortified Hg^{2+} happened to leak into CH_3Hg fraction. However, Hg^{2+} fortification-and-recovery test on CH_3Hg determination would be effective for estimation of validity of the determined CH_3Hg levels in seawater samples being liable to contain sulfides and some others.

Let us refer to the CH_3Hg content in samples A, B and C. Samples A and B of coastal seawater near industrial districts have an unexpected comparatively low content of CH_3Hg. Meanwhile, sample C from rural districts near the hot spring containing mercury has a relatively higher content of CH_3Hg. One of the other reasons for comparatively low content of CH_3Hg in samples A and B is seemingly due to the rapid flux of seawater from the Pacific Ocean into Osaka Bay which dilutes the background contamination. For example, T-Hg of the entrance of Osaka Bay at Kata was 3.2 ppt (Figure 1).

Fujita and Iwashima[1] had reported that in surface seawaters around the Japanese Archipelago, T-Hg nearly 10 ng l^{-1} and CH_3Hg about 1 ng l^{-1} were contained with CH_3Hg/T-Hg ratios of about 10%. In our results, T-Hg in surface seawaters was about 10 ng l^{-1} of which nearly 1% was thought to be the content of CH_3Hg. Estimated T-Hg values by the two laboratories agree well, but not for the CH_3Hg contents. In a previous report,[2] we had speculated that the CH_3Hg/T-Hg ratios in natural water would be below 10%, however, accompanied by the enhancement in the sensitivity of CH_3Hg-detection, the ratios seemingly decreased into 1% levels. Precise determination of CH_3Hg in seawater samples seems to

depend on the high sensitivity and on the low background at detection.

CONCLUSION

1) The preconcentration and high sensitivity that we used in this report are capable of analyzing methylmercury at ppt level in various seawaters.
2) The proportion of methylmercury to the total amount of mercury in coastal seawater was constant (around 1%) both in the Japan Sea and the Japan Island Sea.

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