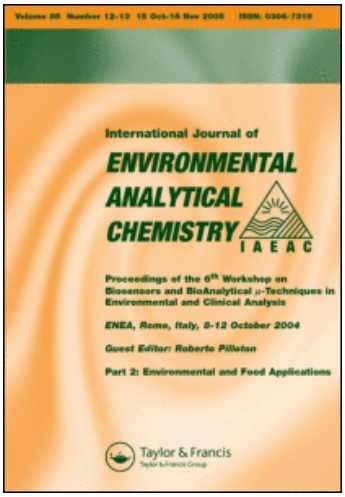

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Determination of Submicrogram Amounts of Inorganic and Organo-Mercury Compounds by Absorption Spectrophotometry

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DETERMINATION OF SUBMICROGRAM AMOUNTS OF INORGANIC AND ORGANO-MERCURY COMPOUNDS BY ABSORPTION SPECTROPHOTOMETRY

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A method for the speciation of inorganic, phenyl and methyl mercury(II) species by spectrophotometry has been developed. Selectivity was achieved by extracting off phenyl and methyl mercury(II) species into benzene and volatilising the inorganic mercury(II) species using stannous chloride for reduction to elemental state. Methylmercury and phenyl mercury(II) were selectively stripped by equilibration with NaOH and EDTA, respectively. The separated species were transformed to mercury paraperiodate by treatment with acidified periodate solution. After masking the unreacted periodate with molybdate, each atom of mercury were made to release 33.2 atoms of iodine through a sequence of reactions. The liberated iodine was transformed to ICl_2^- species by reaction with iodate in the presence of chloride and acid and extracted as ion-pair with rhodamine 6G for spectrophotometric measurement at 535 nm. The method has been applied to natural waters, chloralkali plant effluent, hydrogen gas and biological samples.

INTRODUCTION

The growing interest in understanding the metabolism and toxic effects of trace elements has stimulated considerable interest in the development of methodologies for the quantitative measurement of their various chemical forms at trace levels in environmental samples. Apart from methyl mercury(II) species, which results from biomethylation processes, contamination of the environment by other organomercurials largely occurs due to their production and application as fungicides and pesticides, and in pharmaceutical preparations.

Although a variety of spectrophotometric methods have been described for the determination of mercury, neither the widely used dithizone method[1] nor those based on ion-association complexes[2–6] are sufficiently sensitive for determining the mercury level in environmental samples without resorting to a preconcentration step. In addition, they fail when applied to organomercurials and necessitate their conversion to inorganic mercury(II) compounds prior to determination.[7]

A highly sensitive ($\varepsilon = 5.3 \times 10^5$ L mol^{-1} cm^{-1}) spectrophotometric method based on Liepert amplification reaction through the formation of iodate by action of bromine on HgI_4^{2-} has recently been described.[8]

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The iodine formed was determined after reaction with iodate in the presence of chloride and acid to form ICl_2^- species to facilitate its extraction as ion-pair with rhodamine 6G into benzene for measurement at 535 nm.

$$Hg^{2+} + 4\ I^- \rightarrow HgI_4^{2-} \quad (1)$$

$$HgI_4^{2-} + 12\ Br_2 + 12\ H_2O \rightarrow 4\ IO_3^- + 24\ HBr + Hg^{2+} \quad (2)$$

$$4\ IO_3^- + 20\ I^- + 24\ H^+ \rightarrow 12\ I_2 + 12\ H_2O \quad (3)$$

$$2\ I_2 + IO_3^- + 6\ H^+ + 10\ Cl^- \rightarrow 5\ ICl_2^- + 3\ H_2O \quad (4)$$

It appeared to us that the reaction of mercury compounds with periodate to form mercury paraperiodate can be put to advantage not only to improve the sensitivity but also to make the method applicable to all forms of mercury(II) species because of its favourable potential (1.6 V). The incorporation of this step as an integral part of the procedure was found useful for the determination of $MeHg^+$ and $PhHg^+$ with identical sensitivity and with slight diminution in sensitivity for inorganic mercury(II) compounds.

$$5\ Hg^{2+} + 2\ H_5IO_6 \rightarrow Hg_5(IO_6)_2 + 10\ H^+ \quad (5)$$

$$Hg_5(IO_6)_2 + 34\ I^- + 24\ H^+ \rightarrow 5\ HgI_4^{2-} + 8\ I_2 + 12\ H_2O \quad (6)$$

Prior reduction of inorganic mercury(II) compounds to elemental state, not only enhanced the sensitivity that was identical to that of methyl and phenyl mercury(II) compounds but also improved the selectivity considerably. The results of our study for the quantitative liberation of iodine after selective formation of mercury(II) paraperiodate from inorganic, methyl and phenylmercury compounds to facilitate their determination when present at nanogram levels are presented below.

EXPERIMENTAL

Apparatus

A Carl Zeiss PMQII spectrophotometer with 10 mm quartz cells, a glass vessel [9 cm × 4.5 cm] and a bubbler/trap[10 × 2 cm] both with an inlet tube extending up to 0.5 cm from the bottom and an outlet tube at the top were used. The suction end of compressor was connected to the outlet tube of the bubbler. The outlet of the reduction vessel was connected to the inlet tube of the bubbler using latex tubing (Figure 1).

Reagents

Standard solutions. Stock solutions containing 1000 μg/ml of mercury were prepared as follows:

Mercury(II) solution. Prepared by dissolving 0.1354 g of mercuric chloride in 10 ml of nitric acid and diluting to 100 ml with water.

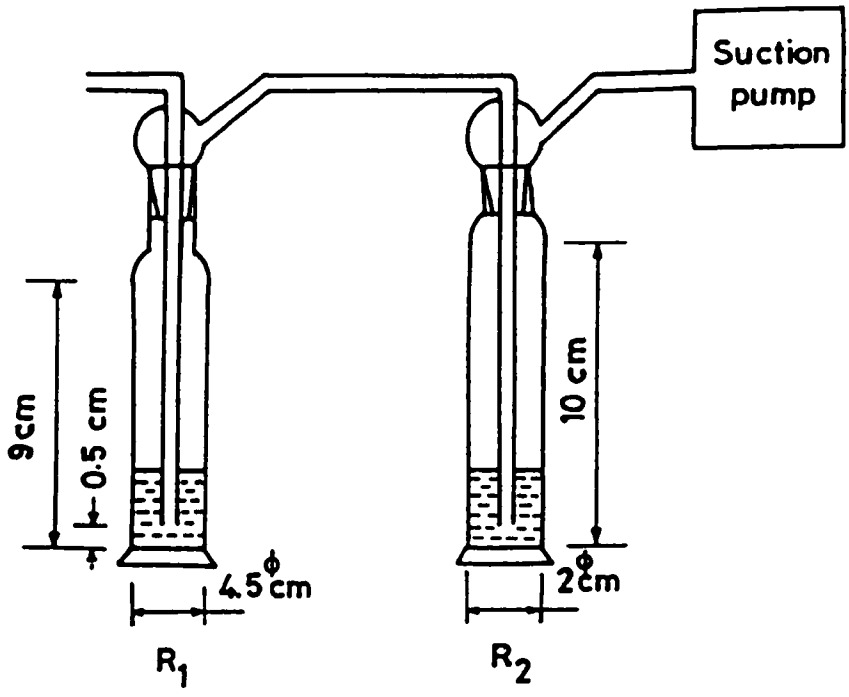

Figure 1 Schematic diagram for trapping elemental mercury. R_1—Reaction vessel (mercury vapour generator) R_2—Trap.

Phenyl mercury(II) acetate solution. Prepared by dissolving 0.1675 g of phenylmercuric acetate in 5 ml of acetic acid and diluting to 100 ml with water.

Methyl mercury(II) chloride solution. Prepared by dissolving 0.1252 g of methyl mercury(II) chloride in 10 ml of HCl and diluting to 100 ml with water. The solution was stored in a refrigerator.

1,10-phenanthroline cadmium solution ($10^{-5}M$). Prepared by mixing aqueous solutions of 0.02 g of 3 $CdSO_4 \cdot 8\ H_2O$ and 0.2 g of 1,10-phenanthroline and diluting to 250 ml.

Potassium periodate solution (0.001%). Dissolve 0.01 g of periodate in 10 ml of 10 N H_2SO_4 and dilute to 1000 ml. Store the solution in an amber coloured bottle.

The following solutions were prepared by dissolving appropriate amounts of the reagents in distilled water:

Sodium acetate, 0.1 M
Stannous chloride, 20% in 10% HCl
Ammonium molybdate, 0.5%
EDTA solution, 0.004 M
Potassium iodide solution, 0.01 N, 0.1 N
Bromine water, saturated

Sulphosalicylic acid, 0.5%
Potassium iodate, 0.01%
Rhodamine 6G solution, 0.02%
Sulphuric acid, 5 N
Benzene (thiophene free) for extraction

Procedure

Formation of mercury(II) paraperiodate

a) Inorganic mercury: Transfer the sample solution not exceeding 50 ml acidified to pH < 1 to the reduction vessel and treat with 2 ml of 20% solution of stannous chloride. Draw air for ten minutes at a rate of 0.5 L/min and collect the elemental mercury in 5 ml of 0.001% solution of periodate in 0.1 N H_2SO_4 contained in the bubbler.

b) Phenyl and methylmercury(II): Treat the sample solution not exceeding 20 ml and containing not more than 250 ng of mercury as phenyl or methyl mercury with 5 ml of 0.001% solution of periodate in 0.1 N H_2SO_4.

Determination

Transfer the periodate solution containing mercury to a 60 ml separatory funnel and treat with 1 ml of 0.5% solution of ammonium molybdate and 5 ml of 0.1 M sodium acetate solution. Mix well and add 2 ml each of 0.1 M solution of KI and 1 N H_2SO_4 and 1 ml of 1.0^{-5} M tris cadmium(II)-phenanthroline solution. Dilute the solution to about 25 ml and equilibrate for two minutes with 5 ml of benzene. Separate the organic layer containing iodine and $HgI_4^{=}$-Cd(II) phenanthroline ion pair and wash twice with 5 ml of water. Discard the aqueous layer and washings.

Treat the organic extract with 5 ml of 0.004 M EDTA and equilibrate for two minutes. Drain the stripping into a 50 ml beaker and treat with sufficient bromine water (pale yellow) followed by 1 ml of 0.5% solution of sulphosalicylic acid. Mix well and add 2 ml each of 0.1 M solution of KI and 1 N H_2SO_4 and dilute to 25 ml. Return the solution to the separatory funnel containing the benzene layer and equilibrate for one minute. Allow the layers to separate and discard the aqueous one.

Wash the benzene layer twice with 5 ml of water and equilibrate with 25 ml of solution containing 2 ml each of 0.01% solution of potassium iodate, 5 N H_2SO_4 and 0.02% solution of rhodamine 6G and 4 ml of 15% solution of NaCl for one minute. Separate the benzene layer into a dry test tube and add about 1 g of anhydrous sodium sulphate. Measure the absorbance of the extract at 535 nm in 10 mm cells against the reagent blank run through the entire procedure. Establish the concentration by reference to a calibration graph prepared using 0 to 250 ng of mercury(II) and following the above procedure.

Separation and determination of inorganic, phenyl and methyl mercury(II) compounds

To the sample solution containing inorganic, phenyl and methyl mercury(II) species, each containing not more than 250 ng of mercury, add 2 ml of 1 N H_2SO_4 and dilute to 20 ml. Transfer the solution to a 60 ml serapatory funnel. Extract the phenyl and methyl

mercury present in solution by equilibrating it twice for two minutes, using 5 ml of benzene each time. Preserve the combined organic phase for the separation and determination of phenyl and methyl mercury(II) species.

Transfer the aqueous phase to the reduction vessel and treat with 2 ml of 20% solution of stannous chloride. Stir for 5 minutes and displace the elemental mercury by drawing air at 0.5 L/min for ten minutes through the solution and collect the displaced mercury in a trap containing 5 ml of 0.001% solution of periodate in 0.1 N H_2SO_4. Complete the determination for Hg(II) species following the procedure described in the previous section.

Strip the combined organic phase by equilibrating with 5 ml of 0.1 N NaOH for one minute. Separate the aqueous phase and treat with 5 ml each of 0.1 N H_2SO_4 and 0.001% solution of periodate in 0.1 N H_2SO_4 and use it for the determination of methyl mercury(II) species following the procedure described under "Determination".

Equilibrate the organic phase containing phenyl-mercury(II) species with 5 ml of 0.004 M EDTA for one minute. Separate the stripping and treat with 5 ml of 0.001% solution of periodate in 0.1 N H_2SO_4 and use it for the determination of phenyl mercury(II) species as described above.

RESULTS AND DISCUSSION

Formation of mercury(II) paraperiodate

Initial studies were performed using 200 ng of Hg^{2+} and 5 ml of 0.001% periodate solution. The solution was adjusted to pH 3.0 and treated with 1 ml of 0.5% solution of ammonium molybdate to mask the free periodate.[9] After adding 2 ml of 0.1 N KI and 1 ml each in 1 N H_2SO_4 and 10^{-5} M Cd(II)-phenanthroline[CPS], the solution was shaken with 5 ml of benzene. The organic layer containing iodine and HgI_4^{2-}-Cd(II)-phenanthroline ion-pair was separated, washed twice with 5 ml of water and subjected to determination as described before.

The variation of the acidity for the formation of mercury(II) paraperiodate is shown in Figure 2. The formation was instantaneous and quantitative in the acidity range 0.1–2 N with respect to H_2SO_4. At acidities greater than 2 N, formation of mercury(II) paraperiodate was incomplete but can be made to go to completion by adding an excess of periodate. As the addition of 3 ml of 0.001% solution of periodate was found sufficient for quantitative formation of mercury(II)-paraperiodate when the acidity was maintained in the range 0.1–2 N, it was decided to prepare a 0.001% solution of periodate in 0.1 N H_2SO_4 and use 5 ml for the formation of mercury(II)-paraperiodate. Subsequent addition of 5 ml of 0.1 M solution of sodium acetate was found sufficient to raise the pH to 3.0 for masking the free periodate with molybdate. Although the addition of 1 ml of 0.35% solution of ammonium molybdate was adequate, its presence in excess of this amount had no effect on the recovery of mercury. Later addition of 2 ml of 0.01 N KI facilitated the formation of stoichiometric amount of iodine by reaction with mercury(II) paraperiodate. Under these conditions the calibration graph was linear up to 300 ng of mercury. The molar absorptivity was found to be 6.6×10^5 L mol^{-1} cm^{-1}.

Figure 3 shows the effect of acidity for the formation of mercury(II) paraperiodate when methyl and phenyl mercury(II) compounds were reacted with periodate. Quantitative formation was found to proceed instantaneously from pH 2 to 2 N and from pH 3 to 2 N with phenyl and methyl mercury(II) compounds respectively indicating that

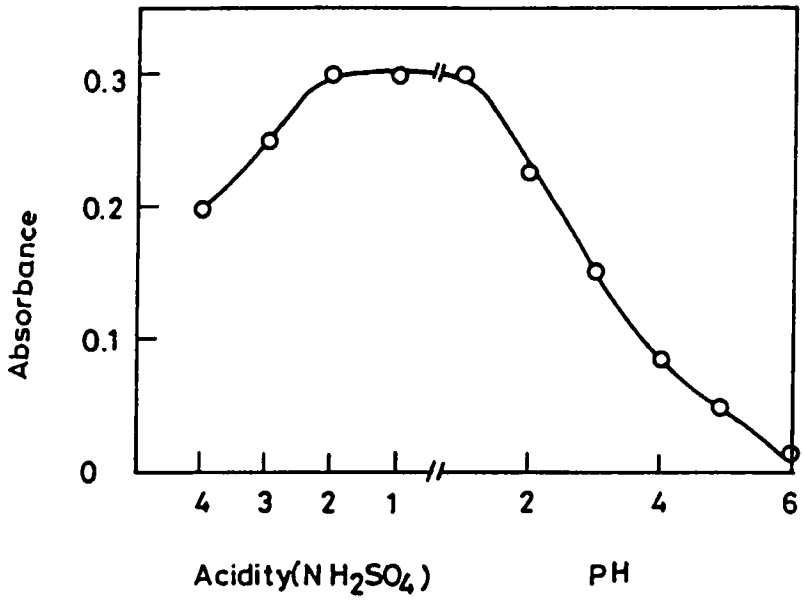

Figure 2 Effect of acidity. Formation of Hg(II)-paraperiodate: Hg(II)—200 ng, 2.5 N H_2SO_4—x mL, 0.001% periodate—5 mL, aqueous volume—10 mL, 10^{-5} M CPS—1 mL, Liberation of iodine: 0.5% ammonium molybdate—1 mL, pH-3.0, 0.1 M KI—2 mL, 1 N H_2SO_4—2 mL, total volume—25 mL, benzene for extraction—5 mL

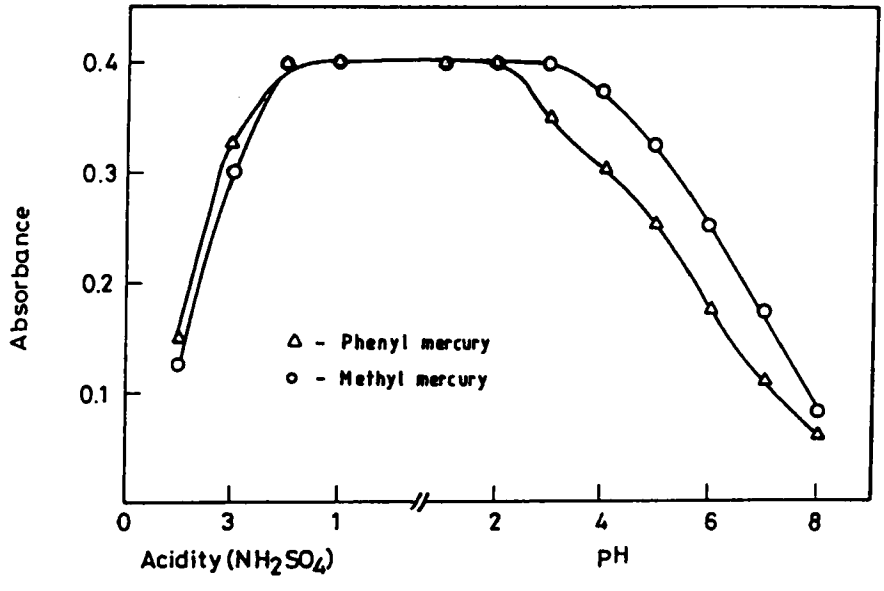

Figure 3 Effect of acidity for the oxidation of phenyl or methyl mercury with periodate Mercury as phenyl or methyl mercury—200 ng, 0.001% periodate—5 mL, 1 N H_2SO_4—1 mL.
Liberation of Iodine: 0.5% ammonium molybdate—1 mL, 0.1 M sodium acetate, —5 mL, 1 N H_2SO_4—2 mL, 10^{-5} M CPS—1 mL, aqueous volume—25 mL, benzene for extraction—5 mL, stripping solution—(0.004 M EDTA)—5 mL, 1 N H_2SO_4—1 mL, bromine water, 0.5% sulphosalicylic acid—1 mL, 0.01 M KI—1 mL, 1 N H_2SO_4—1 mL, benzene for extraction—5 mL.

the acidity range was almost identical to that of inorganic mercury(II) compounds. The absorbance values obtained for phenyl and methyl mercury(II) species containing identical amounts of mercury, however, were found to be 16% higher than that obtained for inorganic mercury compounds. Calibration graphs for both the species, were found to be linear in the concentration range 0–250 ng. The apparent molar absorptivity, as established from the slope of the calibration graphs, was found to be 8.1×10^5 L mol^{-1} cm^{-1} for both phenyl nad methyl mercury(II) species.

Separation and determination of inorganic, methyl and phenyl mercury(II) species

As the reaction of inorganic, phenyl and methyl mercury(II) species with periodate occurred at almost identical acidity, the possibility of their separation by selective extraction was taken up for detailed examination.

The extractability of phenyl and methyl mercury(II) species into benzene[10] in the presence of Hg(II) was investigated using cold vapour atomic absorption spectrophotometry to establish their levels remaining in the aqueous phase.[11–13] As single equilibration with 5 ml of benzene extracted only 90–95% of these species into the organic layers, it was found necessary to repeat the extraction for quantitative recovery as reported elsewhere.[14] Both phenyl and methyl mercury(II) species, as evident from Figure 4, extracted quantitatively when equilibrated twice with 5 ml portions of benzene in the pH range 1–2 leaving the inorganic mercury in the aqueous phase. The extraction remained unaffected up to an aqueous to organic phase volume ratio of 4:1.

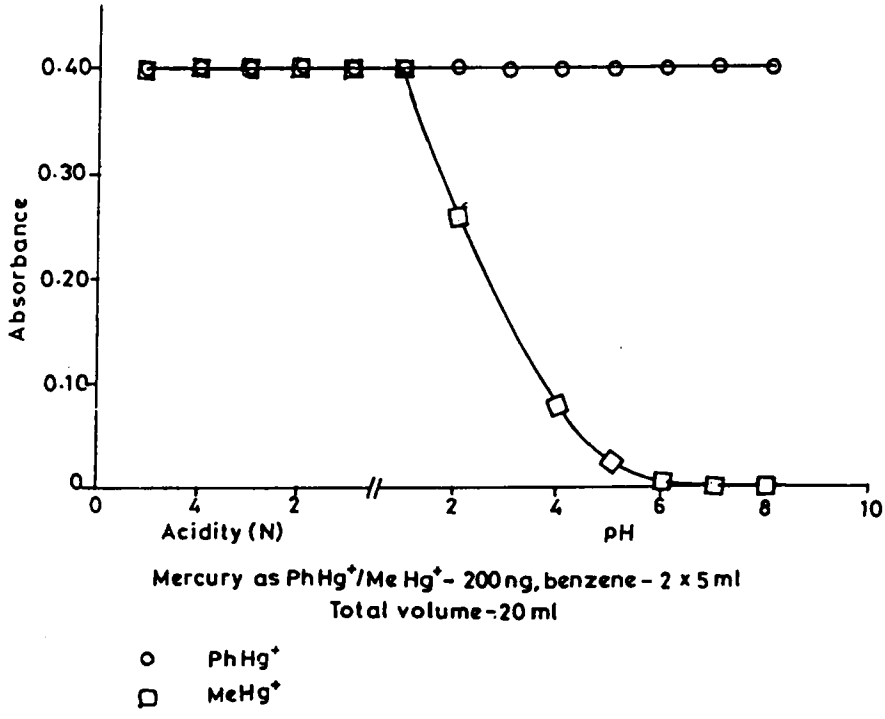

Figure 4 Variation of acidity for the extraction of Ph Hg$^+$/Me Hg$^+$ into benzene.

As the extraction behaviour of phenyl and methyl mercury(II) compounds were similar, the possibility of their separation by selective stripping from the organic layer was examined. The solutions examined included EDTA (0.01 M), NaOH (0.1 N), Na_2CO_3 (0.1 N), $NaHCO_3$ (0.1 N) and NH_3 (0.1 N). While NaOH solution in the range 0.1–0.5 N selectively and quantitatively stripped methyl mercury(II) species, as low as 0.004 M solution of EDTA was found adequate to strip both methyl and phenyl mercury(II) species quantitatively. Stripping of both the species into Na_2CO_3, $NaHCO_3$ and NH_3 were found to be quite incomplete.

Although the aqueous layer containing the inorganic mercury(II) species, after the separation of phenyl and methyl mercury(II) species can be directly subjected to determination, in view of the possibility of interference from Ag(I), Pb(II), Cu(II) and Cd(II), which are known to form paraperiodates, its separation by displacement from the solution after reduction into elemental state with $SnCl_2$ and using periodate solution itself for collection purposes was examined.

The collection efficiency was established by determining the untrapped mercury which escaped through periodate trap after collecting in acidified permanganate[15] using cold vapour atomic absorption spectrophotometry. Studies were done using 200 ng of Hg(II) at an acidity of 2 N for reduction with 20% stannous chloride solution[11]. The elemental mercury formed was displaced by drawing air at 0.5 L/min for ten minutes through a bubbler containing 5 ml of 0.001% solution of periodate with the overall acidity maintained in the range pH 4 up to 5 N with respect to H_2SO_4. The results showed that the recovery of mercury was quantitative when the acidity of periodate solution was in the range 0.04 N–2 N. Under these conditions there was no interference from Ag(I), Pb(II), Cu(II), Cd(II) when present at 1 mg level. However, when the periodate solution after trapping the elemental mercury was subjected to colour development following the procedure evolved, there was about 16% enhancement in the absorbance. The absorbance obtained was identical to that obtained when mercury present as phenyl and methyl mercury(II) species were directly subjected to determination. Evidently, the iodate resulting from the oxidation of elemental mercury by periodate was responsible for the observed enhancement.

Based on these findings a method was evolved for the separation and determination of inorganic, phenyl and methyl mercury(II) species when present together. After extracting off the phenyl and methyl mercury (II) species by equilibrating twice with benzene, the aqueous layer containing inorganic mercury was acidified and treated with stannous chloride. The elemental mercury formed was displaced from the solution into periodate solution and subjected to determination. The combined benzene extract was equilibrated with 0.1 N NaOH to strip the methyl mercury(II) species followed by 0.004 M EDTA solution to strip the phenyl mercury. The strippings were treated with acidified periodate solution to facilitate the formation of paraperiodate and the determination was completed following the procedure evolved for inorganic mercury.

Precision studies

Table 1 gives the results of precision studies based on 10 determinations for 200 ng of mercury when present alone as inorganic, phenyl or methyl mercury(II) compounds. Methyl and phenyl mercury(II) species were determined after extracting into benzene and stripping into 0.1 N NaOH and 0.004 M EDTA respectively. Inorganic mercury(II) was determined after separation as elemental mercury by reduction with $SnCl_2$. The average recoveries were found to be 197.3 ng, 198.7 ng and 198.8 ng for mercury(II),

Table 1 Precision data on the determination of 200 ng of mercury present as Hg^{2+}, $PhHg^{+}$ and $MeHg^{+}$ (for ten determinations)

Compound	*Mean absorbance units*	*Range absorbance units*	*Standard deviation*	*Relative standard deviation (%)*
Hg^{2+}	0.384	0.380–0.410	0.016	4.2
$PhHg^{+}$	0.400	0.390–0.400	0.019	4.8
$MeHg^{+}$	0.380	0.365–0.390	0.015	3.9

phenyl and methyl mercury(II) species respectively. The coefficients of variation were found to be 4.2% for inorganic mercury, 4.8% for phenyl mercury and 3.9% for methyl mercury(II) compounds.

Recovery studies

The results of analysis of mixtures containing varying amounts of inorganic, phenyl and methyl mercury(II) species, each with mercury content not exceeding 250 ng, following the separation and determination procedure given under experimental are furnished in Table 2. The good recovery of each species clearly demonstrates that the proposed method works satisfactorily for the sequential determination of inorganic, phenyl and methyl mercury(II) compounds.

Reaction sequence for the observed enhancement

When 200 ng of Hg(II) was treated with periodate and the determination was completed by following the procedure described under 'Determination' it gave an absorbance of 0.335. In accordance with equations 1 to 6, as 200 ng of Hg(II) would yield 3454.4 ng of iodine and since the absorbance was identical to that obtained when 3.45 μg of iodine in benzene was directly subjected to determination, it was concluded that under the reaction conditions there was stoichiometric formation of mercury(II) paraperiodate.

The inclusion of reduction step with stannous chloride and collection of elemental mercury in periodate as an integral part of the procedure would yield an additional

Table 2 Recovery studies

Sl. No.	*Mercury present (ng)*			*Mercury found (ng)*		
	Hg^{2+}	*$PhHg^{+}$*	*$MeHg^{+}$*	*Hg^{2+}*	*$PhHg^{+}$*	*$MeHg^{+}$*
1	100.0	100.0	50.0	100.0	96.0	48.0
2	50.0	50.0	100.0	46.0	48.0	98.0
3	150.0	50.0	150.0	152.0	46.0	150.0
4	20.0	50.0	175.0	20.0	48.0	175.0
5	100.0	15.0	135.0	100.0	18.0	135.0

175 ng of iodate [due to reduction of periodate by 200 ng of Hg(o)] and hence 762 ng of iodine in accordance with:

$$Hg(o) + IO_4^- + 2\,H^+ \rightarrow Hg^{2+} + IO_3^- + H_2O \quad (7)$$

$$IO_3^- + 6\,H^+ + 5\,I^- \rightarrow 3\,I_2 + 3\,H_2O \quad (8)$$

Accordingly the absorbance increased to 0.400 from 0.335. The enhancement observed (0.065) was identical to that obtained when the iodine formed by directly reacting 175 ng of iodate with iodide was extracted into benzene and subjected to determination. The observed absorbance, in any case, was similar to that obtained with 200 ng of mercury present as phenyl or methyl mercury(II) species when subjected to determination by reaction with periodate. Evidently, with these species, as with elemental mercury, a 2 electron redox reaction results in the formation of iodate in accordance with

$$2\,RHgX + IO_4^- + 2\,H^+ \rightarrow 2\,Hg^{2+} + IO_3^- + 2\,R + 2X^-\,H_2O \quad (9)$$

$$(X = Cl^-, CH_3COO^-)$$

This was confirmed by replacing periodate with peroxide[16] so that oxidation of the organic mercury proceeds without the formation of iodate. The absorbance in this instance was identical to that obtained with 200 ng of Hg(II) when subjected to direct determination without reduction step (0.335) confirming that iodate formed (eqn. 9) was responsible for the observed enhancement.

APPLICATIONS

The method developed was applied to establish the elemental mercury content of hydrogen gas, inorganic mercury content of chloralkali plant effluent, methyl mercury content of biological samples and for the analysis of sea water and tapwater for various mercury species. The results obtained were compared with those obtained by cold vapour atomic absorption spectrophotometry.

Elemental mercury content of hydrogen gas was established by allowing the gas to flow at a rate of 0.5 L/min through a bubbler containing 5 ml of 0.001% solution of periodate in 0.1 N H_2SO_4 for various intervals of time. The periodate solution was analysed by the recommended procedure. The results obtained were compared using 0.5% solution of $KMnO_4$ acidified to 0.9 N with H_2SO_4 for trapping the elemental mercury and analysing the solution, after destroying excess permanganate with hydroxylamine hydrochloride solution by cold vapour atomic absorption spectrophotometry.[15]

Sea water samples collected from Bay of Bengal, Madras coast and effluent water samples from a chloralkali plant were acidified to pH ~1 with H_2SO_4, filtered through a 0.45 pm millipore membrane filter before subjecting to determination.

Biological samples were homogenised with sufficient water, treated with 20 ml of 1:1 HCl:H_2SO_4 mixture and allowed to stand overnight.[17] The extract was filtered, made up to a known volume and analysed.

The results and those obtained for samples spiked with mercury species under study are shown in Tables 3 and 4. It is clear that the proposed method works satisfactorily.

Table 3 Analysis of hydrogen gas

Volume of hydrogen gas passed	*Amount of mercury found*	
(l)	*AAs* (ng)*	*Proposed method (ng)*
2.5	54.0	56.0
5.0	110.0	110.0
7.5	160.0	165.0
10.0	225.0	222.0

* After trapping in permanganate.

Table 4 Analysis of natural waters and effluent samples.

Sample	*Amount added (ng*)*			*Amount found (ng)*			*Recovery (%)*		
	Hg^{2+}	$MeHg^{+}$	$PhHg^{+}$	Hg^{2+}	$MeHg^{+}$	$PhHg^{+}$	Hg^{2+}	$MeHg^{+}$	$PhHg^{+}$
Tap water (20 ml)	–	–	–	30.0	–	–	–	–	–
32.0 ng*	50.0	–	–	80.0	–	–	100.0	–	–
	–	50.0	–	–	52.0	–	–	104.0	–
	–	–	50.0	–	–	48.0	–	–	96.0
	50.0	50.0	–	85.0	42.0	–	106.2	93.3	–
	50.0	–	50.0	78.0	–	48.0	97.5	–	96.0
	–	50.0	50.0	–	50.0	52.0	–	100.0	104.0
Sea water (20 ml)	–	–	–	56.0	–	–	–	–	–
	30.0	–	–	85.0	–	–	98.3	–	–
55.0 ng*	–	30.0	–	–	28.0	–	–	93.3	–
	–	–	30.0	–	–	30.0	–	–	100.0
	30.0	30.0	–	80.0	32.0	–	93.0	106.0	–
	30.0	–	30.0	85.0	–	28.0	98.8	–	93.3
	–	30.0	30.0	–	30.0	28.0	–	100.0	93.3
Chloralkali plant effluent (ml)	–	–	–	95.0	–	–	–	–	–
99.3 ng	50.0	–	–	140.0	–	–	96.5	–	–

* Established by cold vapour AAS.

CONCLUSION

A method to determine inorganic mercury(II), phenyl and methyl mercury(II) species at nanogram levels by spectrophotometry through the formation of mercury(II)-paraperiodate has been developed. Selectivity was achieved by extraction of organic mercury species into benzene followed by reduction-volatilization step to separate the inorganic mercury(II) species. Equilibration of the organic extract with NaOH followed by EDTA stripped the methyl and phenyl mercury(II) species respectively into the aqueous layer selectively and quantitatively. Use of acidified periodate solution for fixing the evolved elemental mercury and for the oxidation of organic mercury compounds facilitated the indirect determination of these species through liberation of iodine with identical sensitivity. The method developed is most sensitive ($\varepsilon = 8.1 \times$

Table 5 Analysis of biological samples.

Species weight taken (g)	*Total mercury present (μg/g)**	*Amount added (μg/g)*		*Mercury found (μg/g)*				*Recovery (%)*	
				Proposed method		*AAS**			
		Hg^{2+}	$MeHg^{+}$	Hg^{2+}	$MeHg^{+}$	Hg^{2+}	$MeHg^{+}$	Hg^{2+}	$MeHg^{+}$
Fish	0.061	–	–	0.017**	0.044*	0.015	0.046**	–	–
1.76		0.050	0.050	0.065	0.045	–	–	97.0	101.0
		0.030	0.020	0.045	0.060	–	–	95.7	93.7
Oyster	0.088	–	–	–	0.085**	–	0.088**	–	–
6.80		0.050	0.050	0.052	0.132	–	–	104.0	97.7
		0.030	0.020	0.030	0.105	–	–	100.0	100.0
Prawn	1.40	–	–	0.013**	1.38**	0.015	1.38**	–	–
2.25		0.050	0.050	0.060	1.40	–	–	95.2	97.9
		0.030	0.020	0.045	1.41	–	–	104.6	100.7
Crab	1.75	–	–	0.068**	1.70**	0.066	1.68**	–	–
1.10		0.050	0.050	0.115	1.73	–	–	97.4	98.8
		0.030	0.020	0.100	1.72	–	–	102.0	100.0
Mussel	0.085	–	–	–	0.087**	–	0.085**	–	–
3.11		0.050	0.050	0.048	0.135	–	–	96.0	98.5
		0.030	0.020	0.030	0.100	–	–	100.0	93.4
Mussel***	8.00	–	–	–	8.00**	–	8.00**	–	–
0.50		0.050	0.050	0.050	8.05	–	–	100.0	100.0
		0.030	0.020	0.030	8.025	–	–	93.3	100.0

* Established by cold vapour AAS (24)
** Average of 3 determinations
*** Collected near the region where chloralkali plant effluent was let into sea.

10^5 L mol^{-1} cm^{-1}) and perhaps the only spectrophotometric method useful to determine individual amounts of inorganic mercury(II), phenyl and methyl mercury(II) species when present together at nanogram levels. The method is rapid and needs no special skill and provides an alternative to the existing chromatographic[18–23] and flameless atomic absorption methods,[24] which are useful to identify and quantify mercury species in environmental samples. Because of favourable oxidation potential of periodate, it should be possible to extend the method for the determination of other environmentally important mercury species too; but further work is needed to ascertain the behaviour of each under the conditions described in this paper and to evolve a satisfactory procedure for their separation.

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