The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

DEVELOPMENT OF A METHOD FOR THE SPECIATION OF MERCURY IN ENVIRONMENTAL SAMPLE ANALYSIS

(Pembangunan Kaedah Bagi Analisis Penspesian Merkuri Dalam Sampel Sekitaran)

M. M. Rahman[1*], M. Awang[1], A. M. Yusof [2], A. K. H. Wood[3], S. Hamzah[3] and A. Shamsiah[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
International Islamic University Malaysia, Indera Mahakota, 25200 Kuatan, Pahang, Malaysia.
[2]Department of Chemistry, Faculty of Science,
Universiti Teknologi Malaysia, Skudai, Johor Bahru, 81310 Malaysia.
[3]Malaysia Nuclear Agency,
Bangi, Kajang, 43000 Malaysia

**Corresponding author: mdrahman@iium.edu.my*

Abstract

Trace concentrations of mercury in water samples were determined by a method involving a simple and rapid procedure connecting the flow-injection inductively coupled plasma mass spectrometry (FI-ICP-MS) and graphite furnace atomic absorption spectrometry (GFAAS) techniques. Mercury vapor, generated by sodium borohydride as the reductant, was stabilized by potassium dichromate ($K_2Cr_2O_7$), then released by controlled heating and detected by FI-ICP-MS. A flow injection sample introduction system with time based injection was used and the sensitivity was found to be proportional to the mass of mercury introduced. Methyl mercury(II) was preconcentrated using the ammonium pyrrolidine dithiocarbamate (APDTC)-chloroform extraction procedure and the chloroform extract was introduced into the graphite tube. A linear calibration graph was obtained for 5-150 ng mercury in 2.5 ml of chloroform extract. Because of the high stability of MeHg(II)-APDTC complexes, it is possible to evaporate the extract in order to obtain a crystalline powder to be dissolved with a few micro liters of chloroform enacting MeHg(II) and Hg(II) can be detected at sub-nanogram levels.

Keywords: Methyl mercury, Flow Injection-Inductively Coupled Plasma Mass Spectrometry, Graphite Furnace Atomic Spectrometry and ammonium pyrrolidine dithiocarbamate

Abstrak

Kepekatan surih merkuri di dalam sampel air diuji melalui prosedur yang mudah dan pantas yang menghubungkan Penyuntikan Aliran Spektrometer Jisim Plasma Gandingan Teraruh (FI-ICP-MS) dan teknik Spektrometri Penyerapan Atom Relau Grafit (GFAAS). Wap merkuri, dihasilkan oleh natrium borohidrida yang bertindak sebagai bahan penurun, telah distabilkan oleh kalium dikromat ($K_2Cr_2O_7$), dan kemudiannya dibebaskan dengan pemanasan terkawal dan dikesan oleh FI-ICP-MS. Sistem pengenalan sampel penyuntikan aliran dengan penyuntikan merkuri berdasarkan masa telah digunakan dan didapati kepekaannya berkadar terus dengan kadar merkuri yang diuji. Merkuri(II) metil dipekatkan terlebih dahulu melalui prosedur pengekstrakan amonium pirolidina ditiokarbamat (APDTC)-kloroform dan ekstrak kloroform tersebut dipindahkan ke dalam tiub grafit. Graf kalibrasi yang linear telah diperolehi bagi 5-150 ng merkuri di dalam 2.5 ml ekstrak kloroform. Dengan kestabilan kompleks MeHg(II)-APDTC yang tinggi, ekstrak dapat disejatkan bagi memperoleh serbuk kristal untuk dilarutkan bersama-sama dengan beberapa mikro liter kloroform agar MeHg(II) dan Hg(II) dapat dikesan pada paras sub-nanogram.

Kata kunci: Merkuri metil, Penyuntikan Aliran Spektrometer Jisim Plasma Gandingan Teraruh, Spektrometri Penyerapan Atom Relau Grafit dan amonium pirolidina ditiokarbamat

Introduction

Mercury is an element that can exist in the environment in both the inorganic and organic forms. Both forms are toxic, but it is the organic form that exhibits extreme biological toxicity [1]. In humans, toxicity due to mercury intake is characterized by gastric pain, vomiting and possible renal failure. With chronic poisoning, mercury can cause injury to the central nervous system and renal damage. A level of 0.5 ppb [2] has also been set as the standard

for mercury in water for human consumption. So the trace elements play an important role in establishing the processes affecting these interactions. As the results detailed studies became available, there is a growing need for reliable measurements of very low concentrations of this element in a variety of samples, especially drinking water that is very important and directly related to human health.

Mercury contamination arises mainly from its industrial uses, such as in the manufacture of Hg cells batteries and Hg discharge lamps. It is also used as a catalyst in converting acetylene to vinyl chloride and acetate. Therefore analytically sensitive techniques are required to detect Hg at very low levels. A number of existing techniques are capable of detecting Hg at very low (ppb) levels but these have associated problems, either because of prohibitive cost or the unusual physical properties of the elements, namely, high vapour pressure and high volatility. Conventional flame atomic absorption spectrometry (FAAS) is relatively insensitive for determining Hg as it is extremely volatile and its residence time in the flame is very short. Mercury has a more sensitive line at 184.9 nm but this line cannot easily be utilized because flame gases and oxygen in the atmosphere interfere strongly at this wavelength and hence the less sensitive 253.7 nm line was to be used [1-3].

The high sensitivity and freedom from spectral interferences exhibited for most elements determined by inductively coupled plasma mass spectrometry (ICP-MS), compared with other atomic spectrometric techniques, makes it a very attractive choice for trace element analysis. Although most elements in the periodic table are ionized with an efficiency of more than 90% in the argon plasma, Hg, with an ionization potential of 10.43 eV is ionized with only 32.31% efficiency [4]. This results in a decrease in the detection power for Hg. In samples containing very low concentrations or samples, which have to be diluted in order to diminish matrix effects, detection capabilities became restricted, making analysis difficult. Preconcentration procedures improve sensitivity, and usually the detection limits are achievable. Flow injection (FI) sample preconcentration methods reduce the risk of contamination since FI systems are closed. This is especially important for the determination of ngL^{-1} concentrations where high blank signals could lead to deterioration in precision of the signal to background ratio and degradation in the detection limit [5]. Thus, a suitable analytical method for measuring trace element speciation is necessary. The atomic spectroscopic methods that are usually used for elemental analysis generally do not distinguish the various species present for each element. However, speciation information can be obtained by solvent extraction separations with element selective detection by atomic spectroscopy [6,7]. Graphite furnace atomic absorption spectrometry (GFAAS) is one of the most attractive detection systems for elemental speciation, because of its excellent detection limits [3].

In a study on drinking water pollution from water treatment plants, Hg determination is performed by a preconcentration step with ammonium tetramethylenedithiocarbamate (ammonium phyrolidinedithio carbamate, APDTC) in chloroform, followed by determination by graphite furnace atomic absorption spectrophotometry (GFAAS). In this work a method for determining low levels of Hg by GFAAS with preliminary pre-concentration using the APDTC-chloroform system is described. Previous works have shown that losses of analyte occurred during the thermal cycle before atomization, but these losses can be reduced by matrix modification [6]. For example, absorbance values can be increased by the addition of sulfide ions to the analyte solution; this effect has been attributed to the formation of stable mercury (II) sulfide at the ashing temperature. The objective of this present work was to develop simple methods for the accurate FI-ICP-MS and GFAAS determinations and the speciation of mercury in aqueous samples at concentrations of ng L^{-1}.

Materials and Methods

In this work, a continuous-flow in situ nebulizer/Hg sample introduction system was coupled with ICP-MS for Hg determination with flow injection (FI) analysis. In this study, a solution containing 1 ng mL^{-1} Hg(II) in 0.5% $K_2Cr_2O_7$ and 0.1 M HNO_3 was selected as the model to optimize the operating conditions of the hydride generation (HG) system. This solution was loaded in the injection loop and injected into the hydride generation (HG) system. Several operating parameters could affect the efficiency of hydride formation. The concentrations of $K_2Cr_2O_7$ and HNO_3 in the injected sample and carrier solution, the concentration of $NaBH_4$, and the volume of the mixing coil were studied to obtain the optimal conditions for the simultaneous determination of these four elements.

Reagents for FI-ICP-MS and GFAAS

All chemicals were of analytical grade. The reducing solution consisting of the following reagents (0.2%, 0.5%, 1.0% and 2%) $NaBH_4$ in 0.05% NaOH (2 g $NaBH_4$ + 2g NaOH pellets/l solution). These solutions were freshly prepared; the 0.5% $K_2Cr_2O_7$ solution was prepared by dissolving 0.5g $K_2Cr_2O_7$ (Fluka) in 100 mL of 1:1(v/v) HNO_3 (65%) (Merck). A 1M hydrochloric acid was used as a carrier solution and it was prepared by diluting concentrated (32%) HCl 1:9 (v/v) with deionized water. A primary standard stock solution 1000 mg L^{-1} was used; secondary stock solution of 10 mg L^{-1} was prepared by dilution and working solutions of 5, 10, 15 and 20 μg L^{-1} was used. Sub-boiling distilled HNO_3 was prepared in a PTFE still. Chloroform (Fluka) of analytical reagent grade (1000 ml) was extracted three times with 50 mL of 1 M nitric acid and stored in a pre-cleaned brown-glass bottle. A 1% (m/v) aqueous solution (100 mL) of APDTC (Fluka Chemicals Co) was extracted with six successive 5 mL portions of purified chloroform, and then stored in a pre-cleaned 100 ml PTFE bottle. Mercury (II) chloride stock solution (1000 mg L^{-1}) was prepared by dissolving (0.1354g) mercury (II) chloride in 10 mL of nitric acid and diluted to 100 mL with water. Methyl mercury(II) chloride stock solution (1000 ng L^{-1}) was prepared by dissolving (0.1252 g) of methyl mercury(II) chloride (OCO - China) in 10 mL of 1:9 (v/v) HCl and diluted to 100 ml with deionized water. The solution was stored in a refrigerator at 4 – 10 ^oC. Mercury working standard solutions (1 mg L^{-1}) of methyl mercury(II) compounds were freshly prepared daily by appropriate dilution of the stock solutions with deionized water.

Sampling

The polyethylene sampling vessel of 1.5 L capacity was acid-cleaned, rinsed with deionised water and then dried. The samples was preserved in acids such as 1% HNO_3 and refrigerated until ready for analysis. Water samples were analysed immediately after acidification and filtration by FI-ICP-MS. Drinking water samples were taken from the Negeri Sembilan and Melaka water treatment plants as shown in Figure 1. Water samples collected from four treatment plants at three different points and a total of 12 samples were collected at different intervals. The samples were filtered through a 0.45 μm Millipore membrane (Whatman) in a laminar flow hood and the filtrate was kept under suitable conditions.

Separation and Determination Procedure of Methyl Mercury Compounds by GFAAS

The water samples collected thought to contain inorganic and organic mercury (mainly methyl mercury) species [8]. A 500 mL portion of filtered water sample was transferred into a separating funnel and 1 M HNO_3 was added in order to adjust the pH to 1.7±0.1. A portion (5 x 500 ng of methyl mercury(II) standard solution in 500 mL water) was added, followed by 2 mL of 1% APDTC solution and 10 mL of purified chloroform. After shaking for 30 s then equilibrating the mixture, the phases were allowed to separate for 5 min. The aqueous phase contains inorganic mercury(II) while the organic phase contains organic mercury Hg(II). About 5.0 mL of the clear extract was transferred into a pre-cleaned glass cylinder. The calibration graph was prepared by plotting the peak height against the amount of mercury added to 500 mL of distilled water. The optimized experimental conditions of GFAAS was achieved with a lamp current of 3 mA, wave length of 253.7 nm, drying temperature of 100 ^oC, ashing temperature of 200 ^oC, atomizing temperature of 2000 ^oC, a period of 3 seconds before the purge gas flow stopped.

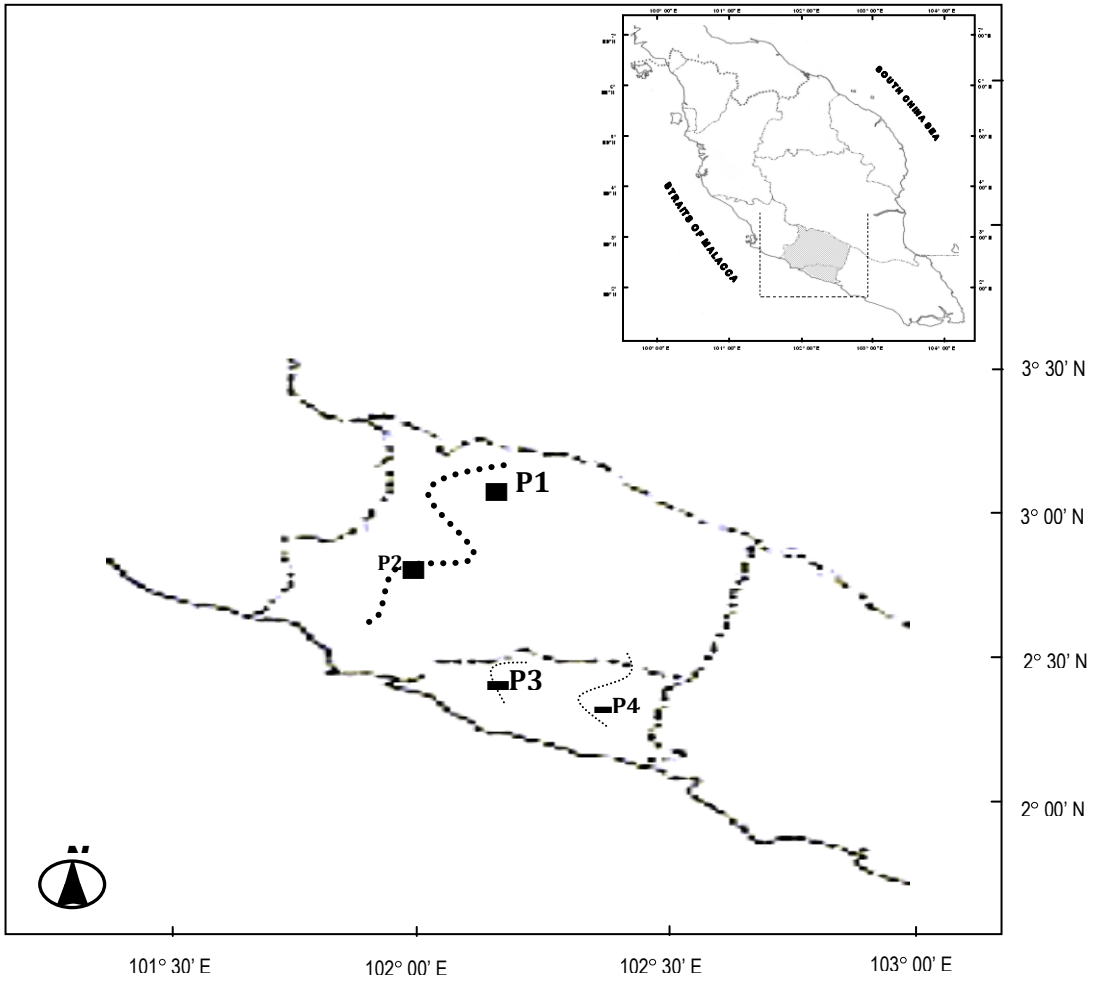

Figure 1. Location of Sampling

P1: Old treatment plant in Negeri Sembilan, P2: New treatment plant in Negeri Sembilan, P3: Old treatment plant in Negeri Melaka, P4: New treatment plant in Negeri Melaka

Results and Discussion

It is well known that the control of accuracy of the results for trace elements in water samples at sub-nanogram levels is extremely difficult. This is not only related to difficulties in the final measurement, but also even more to sampling and handling protocols. Important improvements in sampling and measurement techniques have been achieved, which have resulted in a dramatic increase in the confirmed Hg levels in surface water over the past few years [9]. Samples were kept in the laboratory in PTFE bottles, after they were sampled according to ultra clean protocol. The accuracy of Hg determination in water depends very much on the control of the blanks. These include blanks of the sampling device and sampling handling (storage, preservation, filtration and transportation). However, for this study we could only control blanks from the laboratory processing. Blank values for MeHg were very stable over the period of this study. The instrument detection limit (IDL) was also affected by the reproducibility of blank values. IDL is defined as two standard deviations of the blank. Particularly when low MeHg values were measured, many blanks were run. Another means of confirming the accuracy of the results was to analyze a known concentration standard solution (QCSS). For total Hg determination (0, 5, 10, 15 and 20 ppb) and MeHg (0, 5 and 7ppb) standard solutions were prepared at two different labs, the Malaysia Nuclear Agency Laboratory and

Chemistry Laboratory of University of Technology Malaysia. The verified results from different methods and measured detection limits were shown in the Table 1.

Effect of $NaBH_4$ Concentration on Ion Signals

$NaBH_4$ concentration is critical in the determination of Hg by hydride generation (HG). The effect of the concentration of $NaBH_4$ on the ion signals was investigated first. Figure 2 and Table 2 showed the peak area of the flow injection (FI) peak as a function of $NaBH_4$ concentration. Although the signal of Hg reached a maximum when $NaBH_4$ was about 2%, the Hg signals increased with $NaBH_4$ concentration. For the determination of the Hg, 2% $NaBH_4$ was used in this experiments. In this study, a quite higher concentration of $NaBH_4$ together with lower concentration of NaOH and HCl acid was used for hydride generation (HG). The methyl mercury(II) - APDTC complexes of inorganic and organo-mercury salts are very stable and can be easily recovered in the crystalline form by evaporation of chloroform solutions since the melting points of methyl mercury(II) chloride is 321 ^oC. It is clear that under these circumstances the volume of solvent used determines the smallest amount of Hg detectable. In order to improve the limits of detection, 5 mL of the extract, from a series of aqueous solutions containing 10 ng of mercury as methyl mercury(II) chloride, were evaporated by means of a gentle flow of nitrogen and the residues dissolved with variable but known volumes of solvent. As a result, the absorbance values increase with decreasing solvent volume of 20 μL having been used for the analysis in all examples. It is evident that by employing a preconcentration step a detection limit of less than 1 ng can be achieved.

Table 1. Detection limits obtained by FIMS instrument and GFAAS

Detection limit obtained by FI-MS instrument						
Analyte	QCSS μgL^{-1}	Peak Signal	QC Calcul. Value μgL^{-1}	%RSD	Recovery %	IDL $\mu g\ L^{-1}$
Hg(II)	0.0	0.0014	----	High	----	0.53
	5.0	0.0396	5.01±0.01	2.1	100.1	
	10.0	0.0783	9.98±0.01	2.4	99.8	
	15.0	0.1160	15.03±0.01	3.0	100.2	
	20.0	0.1472	19.99±0.01	5.3	99.9	
Detection limit obtained by GFAAS instrument						
Analyte	QCSS μgL^{-1}	Blank Signal	QC calcul. Value μgL^{-1}	%RSD	Recovery%	IDL $\mu g\ L^{-1}$
MeHg(II)	0.00	0.009	0.15±0.01	17.4	----	0.44
	5.0	0.328	4.96±0.02	0.61	99.2	
	5.0	0.329	4.97±0.04	10.8	99.3	
	5.0	0.330	4.97±0.01	3.4	99.4	
	7.0	0.417	6.41±0.06	14.3	91.6	

IDL: Instrument detection limit, QCSS: Quality Control Standard Sample, %RSD: Relative Standard Deviation

Results of Inorganic Mercury and Methyl mercury

The concentrations of the samples were determined from both the calibrations. The obtained values were reported in μgL^{-1}.

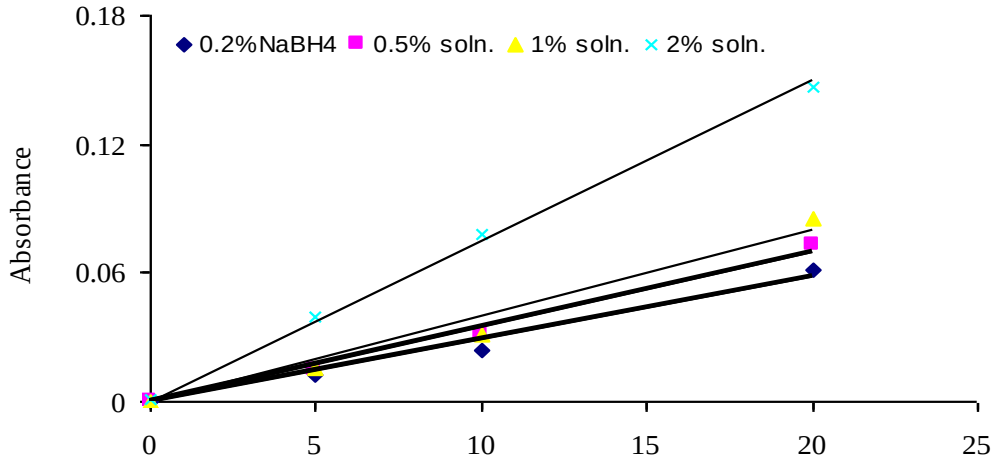

Figure 2. Calibration Curve for different concentrations of $NaBH_4$

Table 2. Obtained absorbance from different $NaBH_4$ solution

Concentration μgL^{-1}	0.2% $NaBH_4$ solution Absorbance	0.5% $NaBH_4$ solution. Absorbance	1% $NaBH_4$ solution. Absorbance	2% $NaBH_4$ solution. Absorbance
Blank	0.0001	0.0001	0.0001	0.0014
5	0.0129	0.0146	0.0153	0.04
10	0.0239	0.0315	0.0316	0.078
20	0.0617	0.0725	0.0858	0.147

The QC data obtained during sample analyses provide an indication of the quality sample data and would be provided with sample results. The results shown in Table 3 show total mercury concentrations in the different water treatment plants in Negeri Sembilan and Melaka (P1 to P4) ranging from 0.60±0.04 μg L^{-1} to 7.11±0.12, while methylmercury levels make up from 18% to 40% of the total mercury. In the raw water taken from the Negeri Sembilan water treatment plants, the total mercury determined were 7.11±0.12 μg L^{-1} and 3.52±0.07 μg L^{-1} from the old and new plant respectively and the methyl mercury ranged between 24.8 μg L^{-1} to 22.7μg L^{-1}, (23~ 25)% of the total mercury recorded. In the raw water (RW) sample taken from the new water treatment plant of Melaka, the total Hg was 5.69±0.02 μg L^{-1} and the methyl mercury recorded was 1.74±0.03 μg L^{-1} representing 30.6% of the total mercury. While the total mercury concentrations were lower in the raw water in old water treatment plants in Melaka, 3.66±0.84 μg L^{-1} (RW-3), the methyl mercury was 31.3% was similar (0.11±0.01 μg L^{-1}) of the total mercury.

Table 3. Determination of mercury and speciation of methyl mercury by FIMS and GF-AAS respectively

SAMPLE ID	Result obtained by FIMS total mercury (μg L^{-1})		Result obtained by GFAAS methyl mercury (μg L^{-1})		Inor. mercury (μg L^{-1})
	Conc.	%RSD	Conc.	%RSD	Conc.
RW-1	7.11±0.12	1.6	1.76±0.015	3.52	5.35±0.12
PW-1	2.81±0.01	0.4	0.58±0.008	2.26	2.23±0.01
FW-1	0.60±0.04	high	> DL	3.42	0.57±0.34
RW-2	3.52±0.07	1.8	0.80±0.01	2.24	2.73±0.07
PW-2	2.21±0.01	0.5	0.78±0.06	9.98	1.43±0.01
FW-2	2.09±0.08	3.7	0.57±0.02	4.32	1.52±0.08
RW-3	3.16±0.84	2.6	0.99±0.02	5.40	2.17±0.84
PW-3	2.14±0.01	0.2	0.93±0.02	3.83	1.21±0.01
FW-3	1.48±0.01	0.6	0.63±0.02	6.02	0.85±0.01
RW-4	5.69±0.02	0.4	1.74±0.03	6.14	3.96±0.02
PW-4	2.69±0.06	2.2	1.11±0.01	1.39	1.58±0.06
FW-4	1.26±0.10	8.0	0.52±0.01	0.87	0.74±0.10

(Total mercury - Methyl mercury = Inorganic mercury); DL: Detection Limit, RW: Raw Water, PW: Pretreatment Water, FW: Fresh Water

While it is premature to draw any conclusive remarks that the differences in the methyl mercury proportion to the total in the two raw water systems, the area could be related to erratic of climate factors, which also affect the characteristics of the biota. A total mercury concentration of 0.60±0.04 μg L^{-1} and 2.09±0.08 μg L^{-1} were found in fresh water at the old (FW-1) and new (FW-2) water treatment plants in Negeri Sembilan. These values compared with the mercury concentrations in the raw water seem to indicate a 91.6 % and 40.6 % of the total mercury removal in the old and new water treatment plants respectively. So it is clear that regarding Hg, the old water treatment plant in Negeri Sembilan was functioning better than the new one. On the other hand, a total mercury concentration of 1.48±0.01 μg L^{-1} and 1.26±0.10 μg L^{-1} were found in fresh water samples (FW-3 and FW-4) at the old and new water treatment plants in Melaka. These values compared with the mercury concentrations in the raw water seem to indicate a 53.2 % and 77.8 % of the total mercury removal in the old and new water treatment plants respectively. From these results, it is clear that the new water treatment plant in Melaka is more efficient than the old plant. Surprisingly the methyl mercury of 0.52-0.63 μg L^{-1} is still present in the clean water samples taken at all the treatment plants, which is supposed to be easily decomposed by chlorine.

Precision and Accuracy

To check the precision of the method (GFAAS), 500-ml portions of distilled water were spiked with 5 x 500 ng of methyl mercury(II) chloride and extracted as already described. The results obtained were listed in Table 1. The difference between the mean mercury content of the drinking water and that of the distilled water represents the amount of mercury in 500 ml of drinking water. The relative standard deviation of seven repeated determinations of 500 mL of distilled water containing 5 ng L^{-1} of mercury (II), as methyl mercury(II) chloride was between 0.61 – 17.4 %.

Reproducibility was determined by using seven injections of a test mixture solution containing 5 μg L^{-1} of Hg. The relative standard deviations of the peak heights for these seven injections were in the range of 2.1 – 5.3 % for the element studied. Calibration curves based on peak heights were linear for the elements studied in the range tested (5 – 20) μg L^{-1}. The detection limits were estimated from these calibration curves based on the usual definition as the amount (or concentration) of analyte necessary to yield a net signal equal to three times the standard deviation of the blank. The detection limit was 0.53 μg L^{-1} for inorganic mercury (Hg).

Conclusion

FI-ICP-MS provides a simple, rapid and accurate technique for the routine determination of trace amounts of Hg in water samples. This method yielded a large signal enhancement factor. Better detection limits would be expected if reagents of higher purity were used. GFAAS method described is extremely simple and allows nano-gram levels of methyl mercury(II) present as organo-mercury salts to be determined in water samples by direct injection of a portion of the chloroform extract. It was found that the chloroform extracts could be stored for more than one week without mercury losses. In order to determine mercury at the sub-nanogram levels it is necessary only to evaporate the chloroform from the extract and to redissolve the residue again with a few microliters of chloroform and to transfer the solution quantitatively into the graphite furnace.

References

1. Ellis L. A. and Roberts D. J., (1996). Novel method to determine mercury in sediment using a gold coated dual 'bent' tube trap. *J. Anal. At. Spectrom.*, 11, 1063-1066.
2. Bunce N., (1994). *Environmental chemistry*, Wuerz Pub. Ltd. Winnipeg, Canada, 2nd Edi., pp., 232-233.
3. Filippelli M., (1984). Determination of trace amounts mercury in sea water by graphite furnace atomic absorption spectrometry." *Analyst, 109*, 515-517.
4. Boomer, D. W. and Powell M. J., (1987). Determination of uranium in environmental samples using inductively coupled plasma mass spectrometry. *Anal. Chem., 59*, 2810-2821.
5. Ebenzer D., Denoyer R. E., and Tyson F. J., (1996). Flow injection determination of mercury with preconcentration by amalgamation on a gold- platinum gauze by inductively coupled plasma mass spectrometry." *J. Anal. At. Spectrom., 11*, 127-132.
6. Krull I. S., (1991). *Trace metals analysis and Speciation*. New York, USA; Elsevier.
7. Ebdon L., Hill S., and Ward R. W., (1987). Directly coupled liquid chromatography - atomic spectroscopy. *Analyst; 112*, 1-16.
8. Schintu. M., T. Kaur and Kudo A., (1989). Inorganic and methyl mercury in inland waters. *Wat. Res., 6*, 699-704.
9. Wan C. C., Chen C. S. and Jiang S. J., (1997). Determination of mercury compounds in water samples by liquid chromatography- inductively coupled plasma mass spectrometry with an in situ nebulizer/ vapor generator. *J. Anal. At. Spectrom, 12*, 683-687.