SOLID-PHASE EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF MERCURY WITH 6-MERCAPTOPURINE IN ENVIRONMENTAL SAMPLES

Mohammad SABER-TEHRANI[(*)1], Hamid HASHEMI-MOGHADDAM[2], S. Waqif HUSAIN [1]and Karim ZARE[1]

[1]Department of Chemistry, Science and Research Branch, Islamic Azad University, P. O. Box 14515-775, Tehran, Iran.
[2]Department of Chemistry, Islamic Azad University, Damghan branch, Damghan, Iran

Summary - A highly selective, sensitive and rapid method for the determination of trace amounts of inorganic mercury based on the reaction of Hg (II) with 6-mercaptopurine and the solid phase extraction of the complex on C_{18} membrane disks was developed. The 6-mercaptopurine selectively reacts with Hg (II) to form a complex in the pH range of 5-8. This complex was preconcentrated by solid phase extraction with C_{18} disks. An enrichment factor of 100 was achieved. The molar absorptivity of the complex is 0.26×10^{-6} L. mol^{-1}. cm^{-1} measured at 315 nm. The Beer's law is obeyed in the concentration range of 0.002 – 0.048 μg mL^{-1}. The relative standard deviation for eleven-replicated measurement of 0.04 μg mL^{-1} is 1.5 %. The detection limit is 0.001 μg mL^{-1} in the water samples. The advantage of the method is that the determination of Hg (II) is free from interference of almost all the cations and anions found in environment and wastewater samples. The determination of Hg (II) in water samples of different origins and marine sediment were carried out by the present method and cold vapor atomic absorption spectrometry (CVAAS). Also the method's accuracy was investigated by using SRM 2709. The obtained results by the present procedure were in good agreement with those of the CVAAS and certified value, so that the applicability of the proposed method was confirmed for the real samples.

INTRODUCTION

Mercury is a serious environmental pollutant with toxic effects in all living organisms.[1] Its affect on the body's immune system is potentially harmful, possibly contributing to diseases such as leukemia.[2] Mercury and its compounds could be present as trace contaminants as a consequence of natural or anthropogenic activities in various environmental samples.[3] It is usually present in natural waters at trace levels.[4] The lakes, rivers and coastal waters in vicinity of the industries that are utilizing mercury in production are essentially the important indicators

(*) Corresponding author; fax: (++98)-021-44817146, e-mail: drmsabertehrani@yahoo.com

of environmental pollution. The development of analytical methods for the determination of mercury is still a challenge.[5]

A serious problem encountered in the determination of mercury is that target species are usually present in low concentration.

The main species of mercury in natural waters to be identified and determined are inorganic mercury (Hg^{2+}) and methyl mercury (CH_3Hg^+). Recent reports estimate total mercury concentration in natural waters ranging from 0.2 to 100 ngL^{-1}, while methyl mercury levels are much lower (ca. 0.05 ngL^{-1}).[6]

The routine spectrophotometric methods are often not sensitive or selective enough to determine low concentration of mercury ion in environmental samples. Consequently, a preconcentration step is usually required. The most widely used preconcentration methods are coprecipitation [7], ion exchange[8], solvent extraction[9,10], flotation[11,12] and solid phase extraction (SPE)[13-16]. Solid phase extraction is an attractive technique that reduces solvent consumption and exposure, disposal costs and extraction time for sample preparation.[17]

6-MercaptoPurine (6-MP) is a biologically active molecule containing sulfur and nitrogen donor sites that can form stable complex with mercury.[18-20]

This study describes a procedure for the determination of inorganic mercury in environmental samples using the solid phase extraction technique. Several significant advantages of the present method include simplicity of the operation, few interferences, excellent detection and avoiding the use of harmful organic solvents.

EXPERIMENTAL

Apparatus

A UV-2101PC Spectrophotometer (Shimadzu, Japan) was used for all absorbance measurements with a 1cm quartz cell.

Solid phase extractions were conducted by C_{18} membrane disks, ENVI-18DSK™ [47 mm (diameter) ×0.6 mm (thickness) 30 µm (particles), 70 Å (pore size)] obtained from Supelco (Bellefonte, USA), in conjunction with a standard Millipore 47 mm filtration apparatus equipped with a desktop vacuum pump. A pH meter Metrohm 744 A model was used for pH measurements. An ultrasonic processor (Unique, Brazil) equipped with a 4mm diameter titanium tip was used. Ultrasonic vibration at the probe tip was fixed at any desired power level using a power setting in the 20–100% range. The ultrasonic processor was enclosed inside a sound proof chamber during operation. The time of sonication could be increased in steps of 5 s each of the intended period of sonication. A small box made of polistyrene foam containing small ice rocks was used as ice bath. When necessary, the sample vessel was inserted in the ice bath so that the temperature did not exceed 30 ^{0}C during sonolysis.[21]

Materials and Solutions

Analytical reagent grade chemicals were employed for the preparation of all solutions. Solutions were prepared using deionized water from a Nanopure water system with specific resistivity of 18.3 MΩ cm^{-1} (Millipore corporation, USA). Methanol from Merck (Darmstadt, Germany) was used. A stock solution of mercury (1000 µg mL^{-1}, Hg (II) in 0.5 mol L^{-1} HNO_3) was prepared from mercury chloride (Merck). Working Hg (II) standards were prepared daily by appropriate dilution of the stock solution. The selected reagent, 6-MP, was provided by Sigma-Aldrich (Steinheim, Germany). A solution of 1.0 $\times 10^{-3}$ mol L^{-1} 6-MP was prepared daily by dilution with the buffer solution. The certified reference material SRM 2709 was obtained from the National Institute of Environmental Studies.

The 0.1 mol L^{-1} phosphate buffer solution was prepared by dissolving appropriate amount of Sodium dihydrogen phosphate in 500 mL water, then adjusting the pH to 6 with sodium hydroxide solution and diluting to a volume of 1000 mL with water.

Special care was taken in the preparation and handling of solutions and containers to minimize any possible risk of mercury contamination. Calibration flasks were left overnight in 10% (v/v) HNO_3 and then rinsed thoroughly with ultra-pure milli-Q water before use to minimize exogenous metal contamination.

General Procedure

To a standard or sample solution containing no more than 12 μg of Hg (II) in 250 mL of a calibrated flask, 25 mL of Sodium dihydrogen phosphate-disodiumhydrogen phosphate buffer solution (containing 0.1 mol L^{-1} Na_2EDTA) and 5 mL of 1.0×10^{-3} mol L^{-1} of 6-MP solution were added. The mixture was diluted to the volume of 250 mL and mixed well. After 10 min, the solution was passed through the C_{18} disk at flow rate of 50 mL min^{-1}. The mercury complex was retained on the disk. After the enrichment was finished, the complex was desorbed from the disk with 2.5 mL of methanol (contain 0.5% KOH) at the flow rate of 5 mL min^{-1} in reverse direction. The absorbance of this solution was measured at 315 nm in a 1 cm cell against a blank reagent prepared in a similar way without mercury.

CVAAS Analysis

The CVAAS analysis was carried out with a Varian (Spectra AA-220) Atomic Absorption Spectrometer equipped with mercury hallow cathodic lamp and a vapor generator accessory (VGA 77) in a continuous system. The experimental conditions were: slit width, 0.5 mm; lamp current, 4 mA; wavelength, 253.7 nm; time constant, 5s; PMT voltage, 290 V

Extraction of mercury from marine sediment

The marine sediment samples were analysed as received, except being ground in an agate mortar in order to obtain particles size ≤77 or ≤121μm. The moisture of these samples was determined and corrected in order to obtain the real sample masses as specified in the certificates. The real samples were dried at ambient temperature in a clean room and then ground in an agate mortar in order to obtain particles size ≤121μm. Then 1.000 g of sample was weighed accurately and transferred into a screw capped 50 mL polypropylene volumetric flask. Afterward, 10 mL of HNO_3 30% (V/V) was added and the flask was left to stand for 30 min. The final slurry was sonicated by using a probe which was inserted into the flask. After the sonication procedure, the slurry was centrifuged during 2 min at 2700 rpm. The supernatant was transferred and neutralized by NaOH. The experimental conditions were: time of sonication 120 s; Ultasonic power, 70 W.

RESULT AND DISCUSSION

Absorbance Spectra

The absorption spectra of 6-MP and its Hg (II) complex under the optimum conditions are shown in FIG. 1. As can be seen, the spectra of the Hg (II)-6-MP complex have two maxima that overlap with the maximum of the ligand. However, it does not interfere in determination of mercury because the unreacted 6-MP would not be retained on the C_{18} disk. The peak at 315 nm is more practicable in real sample. Thus, the wavelength of 315 nm was used in all subsequent absorbance measurements.

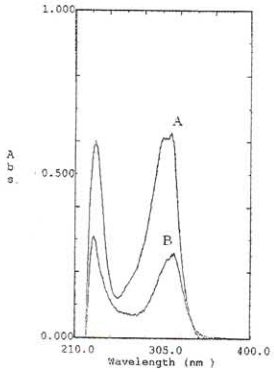

FIGURE 1. - Absorption spectra of 6-MP and its mercury complex (A) Hg (II)-6-MP complex against reagent blank (B) 6-MP in Methanol (contain 0.5%KOH)

Effect of the pH

Our results indicated that the optimal pH for the reaction of Hg (II) with 6-MP was 5.0-8.0, FIG. 2. In acidic pHs formation of complex between Hg (II) and 6-MP is not fast enough and in basic pH the complex would be solved easily in aqueous medium. In acidic pH the selectivity is improved noticeably, therefore, the pH=6 was selected as the optimum.

A Sodium dihydrogen phosphate-disodium hydrogen phosphate buffer solution of pH=6.0 was recommended to control pH. The use of 10-50 mL of the buffer solution (pH=6.0) per 250 mL of the final solution was found to give the maximum and stable absorbance. The use of 25 mL of the buffer solution is recommended.

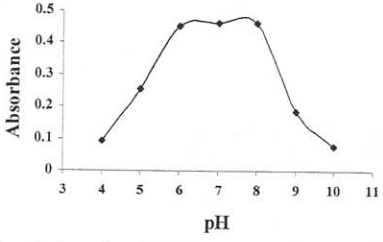

FIGURE 2. - Effect of pH on the formation of Hg(II) complex, Hg(II)

Effect of 6-MP Concentration

The Optimum amount of 6-MP for the quantitative extraction of Hg (II) was also investigated (FIG. 3). From these results, the addition of about 5.0 mL of 1×10^{-3} mol L^{-1} of 6-MP solution has been found to be sufficient for a complete reaction. Accordingly, 5.0 mL of 6-MP solution was added in all further measurements.

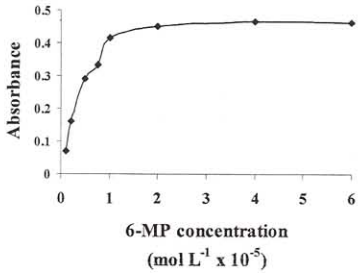

FIGURE 3. - Effect of 6-MP concentration on the determination of Hg(II) by the solid phase extraction method. The pH of the solution was adjusted to 6.00 and $C_{Hg} = 0.024\mu g\ mL^{-1}$ in all the experiments.

Stability of complex

After mixing the reactants, the absorbance reaches its maximum within 6 min at room temperature and remains stable for 12 h in aqueous solution. The complex is stable for at least 24 h if extracted into the methanol (containing 0.5 % KOH).

Effect of Surfactant

The effect of surfactants was tested on the recovery of extraction by a cationic surfactant (CTAB), an anionic surfactant (SDS) and a non-anionic surfactant (Triton- X-100). None of them increase the absorbance markedly. Therefore, no surfactants were added in the final procedure developed.

Solid Phase Extraction

Some experiments were carried out in order to investigate the retention of 6-MP and its Hg (II) complex on the disks. It was found that the Hg (II)-6-MP complex was retained on the disks quantitatively when it passes the disk as aqueous solution. The capacity of the disk for the Hg (II) - 6-MP complex was determined as 18 mg in 250 mL of solution. In this experiment, the disks have adequate capacity to enrich the Hg (II) - 6-MP complex.

In order to choose a suitable eluent for the retained Hg (II) - 6-MP complex, various organic solvents were examined. It was found that pure organic solvents could not elute the Hg (II) - 6-MP complex from the disk quantitatively. Regardless of two types of sites of 6- MP (N,S), it acts as a monodendate ligand through sulfur with mercury[22], therefore the N-H site is

inactive in case of mercury. Moreover, in basic medium, the polarity of complex increases owing to H releasing. Therefore, the solubility of complex increases in polar organic solvents like methanol. So, methanol (containing 0.5% of KOH) was selected as eluent. Experiments showed that it was easier to elute the retained complex in reverse direction. Methanol (2.5 mL containing 0.5% of KOH) was sufficient to elute the complex from the disk at flow rate of 5 mL min^{-1}. Therefore, the volume 2.5 mL of eluent was chosen.

Calibration Curve and Sensitivity

The calibration curve showed that the Beer's law is obeyed in the concentration range of 0.002-0.048 μg Hg(II) per mL in the measured solution. The linear regression equation obtained was: $A = 0.0189 \times 10^3 C$ (μg mL^{-1}) + 0.0076 (r = 0.9919). The molar absorbivity was calculated to be 0.26×10^{-6} L. mol^{-1}. cm^{-1} at 315 nm. The relative standard deviation at a concentration level of 0.4 μg Hg (II) per mL (11 replicate determinations) was 1.5%.

Interferences

The effects of foreign ions were studied by introducing several ions with the various concentrations to the solutions containing 1.2×10^{-7} M of Hg(II) ions. Effect of them was considered as an interfering agent, when the analytical signal exhibited a deviation more than ± 5%; the results are tabulated in TABLE 1. According to these results, the method presents high selectivity. The interferences of Cu, Pb and Cd were eliminated successfully by the use of EDTA. Furthermore, the formation of Ag (I) - 6-MP complex could not interfere in the determination of mercury, because the molar ratio of Ag complex is 1:1 while that of mercury is 1:2. Therefore, the Ag complex would not be retained on the C_{18} disk. In almost all spectrophotometric methods for determination of Hg (II), Ag (I) is a serious interfering cation, but this method presents a selective spectrophotometric method for determination of Hg (II) without Ag (I) interference.

TABLE 1. - Tolerance limits for foreign ions in 1.2×10^{-7} M Hg (II) in a 250-mL solution (relative error ±5%)

Ions	Mole ratio of interfering ion to Hg(II)
Na^+, K^+, F^-	10^7
CH_3COO^-, SO_4^{2-}, PO_4^{3-}	10^6
Ca^{2+}, Mg^{2+}	10^5
Ba^{2+}, Bi^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Cr^{3+}, Co^{2+}, Fe^{2+}, Al^{3+}, Cl^-	10^4
Pb^{2+}, Cd^{2+}, CO_3^{2-}, Cl^-, Ag^-, Br^-	10^3
CN^-	10^2
I^-	1

Application

In order to validate the methodology, the proposed method was applied to different environmental samples for mercury determination. The wastewater and seawater were collected from Ravand factory and Caspian Sea, respectively. The Ravand factory produces sodium hydroxide by electrochemical method. Moreover, the ground water was collected from Abask, that is located in the Damavand range in north of Tehran. The water samples were acidified by HNO_3 and filtered through 0.45 μm millipore membrane filters, prior to analysis. Along with the

samples, several known amount of Hg (II) were spiked to examine the reliability of the method. Marine sediments were collected in an offshore area at Zibakenar, Iran. The validity of the proposed method was confirmed by comparing the results obtained from the sample analysis with those obtained by CVAAS. Also the accuracy of the method was investigated by analysis of soil reference material (SRM 2709) by solid phase extraction. The results of various sample analysis are tabulated in TABLE 2 and TABLE 3.

TABLE 2. Determination of mercury in water samples.

Sample	Hg (II) added ng mL^{-1}-μg g^{-1}	Measured / ng mL^{-1}-μg g^{-1}	
		Proposed method	CVAAS
Waste Water	0	5.20 ± 0.11	5.12 ± 0.07
	16	21.3 ± 0.17	21.16 ± 0.05
	32	37.4 ± 0.15	37.1 ± 0.06
Sea Water	0	N.D.	N.D.
	16	16.14 ± 0.14	16.02 ± 0.04
	32	32.18 ± 0.13	32.04 ± 0.09
Ground Water	0	N.D.	N.D.
	16	16.17 ± 0.09	16.08 ± 0.03
	32	32.18 ± 0.14	32.07 ± 0.06
Marin Sediment	0	N.D.	N.D.
	1.60	1.63 ± 0.09	1.61 ± 0.09
	3.20	3.24 ± 0.14	3.23 ± 0.08

The results are reported as the average value from five sample measurements.
N.D. : Not detected

TABLE 3. - Analysis of certified reference material, expressed as $\overline{X} \pm \left({}^{(tSD)}/_{\sqrt{N}} \right)$ for N = 5 measurements and $t_{(N-1=4)} = 2.78$

	Certified mercury (μg g^{-1})	Found mercury (μg g^{-1})
soil reference material (SRM 2709)	1.40 ± 0.80	13.65 ± 0.28

CONCLUSIONS

The proposed SPE method is a simple, rapid and high selective method for separation, preconcentration and determination of mercury in different environmental samples. Practically none of applied cations interfere with the proposed method; this showed that the complexing agent is very selective toward Hg (II) in presence of other metal ions. This could be considered as an important advantage of both the ligand and the proposed method. The working pH of this method is actually suitable for natural waters. Furthermore, the enrichment factor of 100 was achieved with solid phase extraction by C_{18} disks. The detection limit of proposed method reaches 0.001 μg L^{-1}, therefore the low concentration of mercury could be determined in water samples with good results.

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