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Solid phase extraction and determination of sub-ppb levels of hazardous Hg^{2+} ions

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Abstract

A simple, rapid and reliable method has been developed to selectively separate and concentrate ultra trace amounts of mercury(II) ions from aqueous samples for its highly sensitive measurement by cold vapor atomic absorption spectrometry (CV-AAS). The Hg^{2+} ions were adsorbed selectively and quantitatively during the passage of aqueous samples through octadecyl silica membrane disks modified by isopropyl 2-[(isopropoxycarbothiolyl)disulfanyl]ethane thioate (IIDE). The retained Hg^{2+} ions were then stripped from the disk with minimal amounts of 0.5 M hydrobromic acid (two 8 ml portions) as eluent, and determined by CV-AAS. The break-through volume of the method is greater than 3000 ml, which results in enrichment factors >150. Maximum capacity of the membrane disks modified with 10 mg of the ligand was found to be 350 ± 30 μg of mercury(II), and the limit of detection is 0.005 ng ml^{-1}. The effect of various cationic interferences on the recovery of mercury in binary mixtures was studied. The method was applied to the recovery of Hg^{2+} ions from different synthetic and tap water samples, as well as the determination of mercury in human hair samples.

Keywords: Hg^{2+}; Octadecyl silica disks; SPE; CV-AAS; Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl] ethanethioate

1. Introduction

The toxic effects of mercury are well known. Mercury may enter a human body by inhalation of mercury vapor (mainly in the form of Hg^0), drinking water (mainly as inorganic mercury, Hg^{2+}), and/or by the consumption of fish and fish products (mainly as methylmercury, CH_3Hg^+) in the diet [1]. The contents of mercury species in hair may represent the cumulative exposure from the occupational environment and/or daily diet [2]. Although the levels of total mercury in hair for a normal person are in the range of 0.4–6.0 μg g^{-1}, a concentration greater than 50 μg g^{-1} is considered to be poisonous [3]. The level of total mercury in hair is about 300-times larger than that in blood and a hair sample is easier to acquire and store than one of blood [2]. Hence, the concentration of mercury species in hair would be convenient as a biological marker [2]. However, the direct determination of (ultra) trace amounts of mercury in hair, water and the other complicated matrices is usually difficult owing to matrix interferences and/or insufficient detection power. Consequently, a preliminary pre-concentration and/or separation is usually required.

Several methods commonly used for the pre-concentration of mercury include concentration in a palladium-coated graphite tube [4], pre-concentration on a gold amalgamator prior to using CV-AAS [5], pre-concentration in a liquid nitrogen trap and solid phase extraction [6,7]. Moreover, the solid phase extraction (SPE) cartridges and disks modified by suitable ligands have been successfully used for the selective separation and determination of metal ions [8–11].

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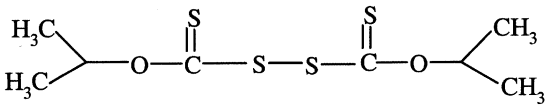
Solid phase extraction (SPE) techniques are now routinely applied in different analytical [12], chromatographic [13], clinical [14], pharmaceutical [15], environmental [16], industrial [17] and agricultural [18] fields. SPE is always used and followed by a direct analytical method for separation and detection of the solid phase extracted organic or inorganic species for the final qualitative and quantitative evaluation procedures. The method provides several major advantages over the classical liquid extraction technique. These include (i) the fast, simple and direct sample application in small size without any sample loss, (ii) no waste generation as practiced in liquid extraction method, (iii) the possibility of interfacing with major chromatographic techniques either in on-line or off-line modes and, finally, (iv) time and cost saving. The analysis of complex samples (e.g., environmental and biological samples) generally involves a pre-treatment step aimed at the reduction of the matrix content and the enrichment of the analyte. This is often performed by solid phase extraction [19].

The aim of this work was the development of a rapid and efficient method for the selective extraction, concentration and CV-AAS determination of $\mu g\,l^{-1}$ levels of Hg^{2+} ion in aqueous solutions and digested hair samples by sorption on octadecyl silica membrane disks modified by isopropyl 2-[(isopropoxycarbothioyl)disulfanyl]ethane thioate (IIDE).



IIDE

2. Experimental

2.1. Reagents

All acids and salts used were of the highest purity available from Merck chemical company and used as received. All organic solvents used were of HPLC grade from Merck. Analytical grade tin chloride, sodium hydroxide and EDTA were purchased from Merck and used as received. Reagent grade mercuric chloride and the nitrate salts of the other cations used (all from Merck) were of the highest purity available and used without any further purification except for vacuum drying. Isopropyl 2-[(isopropoxycarbothioyl)disulfanyl]ethane thioate was synthesized and purified in our laboratories. Doubly distilled, deionized water was used throughout. The standard solution of mercury(II) was prepared by dissolving an appropriate amount of mercuric chloride in 1% (v/v) nitric acid solution. Working solutions were prepared by appropriate dilution of the stock solution with water.

2.2. Synthesis of IIDE

Iodine (1 mmol) in CH_2Cl_2 (10 ml) was added to a stirred solution of potassium *o*-isopropyl(dithiocarbomate) (1 mmol) in CH_2Cl_2 (10 ml) and stirred for 1 h. The reaction mixture was washed with 10% aqueous $Na_2S_2O_3$ (2 ml × 10 ml) and H_2O (2 ml × 10 ml). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. More purification was achieved by re-crystallization in hexane, so that pale yellow crystals of IIDE were obtained in 90% yield (0.24 g). The structure and purity of IIDE was confirmed by elemental analysis, NMR and IR spectroscopy. ^{1}H NMR (CCl_4). δ (ppm): 1.43 (*t*, 12H, CH_3), 5.63 (m, 2H, CH). IR (KBr). ν_{max} (cm^{-1}): 2979.8 (s), 2869.9 (w), 1463.9 (s), 1442.7 (s), 1373.0 (s), 1271.1 (s, b), 1145.6 (s), 1082.2 (s), 1048.0 (s, b) 898.8 (s), 796. 5 (s), 690. 5 (m).

2.3. Apparatus

The determination of mercury was carried out with a Shimadzu AA-670 atomic absorption spectrometer equipped with a Hg-hollow cathode lamp (HCL) and an on-line cold vapor generation system using $SnCl_2$. The absorbance wavelength was set at 253.7 nm (resonance line) and the spectral bandwidth at 0.5 nm. A long path quartz cell (2-cm i.d., 10-cm long) connected to the spectrometer was used as a detection system. The determination of all other cations were carried out with a Shimadzu AA-670 atomic absorption spectrometer under recommended conditions for each metal ion. A digital pH meter, Metrohm model 632, equipped with a combined glass calomel electrode was used for the pH adjustments. Extractions were performed with 47 mm diameter × 0.5 mm thickness 3 M Empore^{TM} membrane disks containing octadecyl-bonded silica (8 μm particle size, 60 Å pore size) distributed by Varian with a standard Millipore 47-mm filtration apparatus.

2.4. Sample extraction

To remove all contaminants arising from the manufacturing process and environment and to ensure optimal extraction of the analyte of interest, disk cleaning and conditioning should carry out before its use. Thus, after placing the membrane disk in the filtration apparatus, 10 ml of methanol were poured onto the disk and immediately drawn through the disk by applying a slight vacuum. The disk conditioning then began by pouring 10 ml acetonitrile onto the disk. Immediately, a low vacuum was applied and the solvent was drawn through the disk. The disk was then dried under vacuum for few minutes. Then, a solution of 10 mg of IIDE ligand dissolved in 4 ml of acetonitrile was introduced onto the disk so that the solution was spread on the whole disk surface, and was drawn slowly through the disk by applying a slight vacuum. This solution was collected in a test tube. A few drops of water were added to the test tube (until just before appearance of a colloidal suspension) and the resulting mixture was again introduced to the reservoir and passed slowly through the disk. The filtration was repeated (if necessary) several times. Finally, the disk was washed with 15 ml water and dried under vacuum for several minutes. The membrane

disk modified by IIDE is now ready for sample extraction. It should be noted that the modified disk thus prepared can be kept at room temperature for over a week, before its use for the extraction of mercury ions.

Sample solutions containing sub-μg amounts of Hg^{2+} are passed through the modified disk at a flow rate of 10–80 ml min^{-1}. The disk was dried by passing air through it. A test tube was then placed under the extraction funnel and the extracted mercury was stripped from the membrane disk by two 8 ml portions of 0.5 M HBr solution at a flow rate of 10 ml min^{-1}. The extracted mercury was transferred to a volumetric flask, diluted to 20-ml with 10% (v/v) nitric acid and the mercury concentration was determined by CV-AAS.

2.5. Determination of Hg^{2+} in human hair samples

The general procedure for the determination of mercury in human hair samples are as follows. The human hair samples were collected from healthy people in the age range of 15–54 years. Each sample (about 10 g) was washed with warmish doubly distilled deionized water, immersed in Triton X-100 (1%, v/v) for about 1 h, washed with doubly distilled deionized water (about 3 l) and then dried in an oven at 105 °C for 3 h. After cooling to room temperature, the hair sample was cut into small pieces (3–5 mm long) by using a pair of stainless-steel scissors, and kept in a capped glass bottle ready for use.

An amount of 400 mg of each hair sample was placed in a 25-ml beaker with a cover. In the case of the hair sample used for recovery tests, an appropriate amount of mercury (0–2 μg, using a 10 μg ml^{-1} Hg^{2+} solution) was also added to the hair sample by a microsyringe. A cover was put on the beaker and the sample was allowed to stand at room temperature overnight to let the solvent evaporate and the mercury remain on the hair. After soaking each hair sample with 2.5 ml of concentrated nitric acid, it was allowed to stand at room temperature for several minutes. Afterwards, the beaker was placed in a larger beaker containing some water, for temperature control, and heated in an oven at 90 °C for 1–1.5 h for complete digestion of the sample. After cooling to room temperature, the pH of the digested sample solution was adjusted to 5–6 using NaOH. The sample solution was then passed through a sintered glass funnel by applying vacuum. After the filtration was finished, the sintered glass was eluted with water, and quantitatively collected. The resulting sample solution was passed through a modified membrane disk and the recommended procedure for determination of mercury was carried out.

3. Results and discussion

Based on the well known hard-soft acid–base theory [20,21], the existence of four donating sulfur atoms in the flexible structure of IIDE was expected to increase both the stability and selectivity of its Hg^{2+} complex over other metal ions including alkali, alkaline earth and many transition and heavy metal ions.

Table 1
Recovery of mercury from the modified membrane disk using different stripping solutions[a]

Stripping solution	Recovery (%)		
	First 8 ml	Second 8 ml	Total
KSCN (1 M)	2.5	1.0	3.5
KBr (1 M)	20.0	6.7	26.7
EDTA (0.25 M)	0.0	0.0	0.0
EDTA (1 M)	25.0	2.8	27.8
HNO_3 (1 M)	0.0	0.0	0.0
HCl (2 M)	65.2	8.7	73.9
HBr (0.10 M)	15.0	4.5	19.5
HBr (0.50 M)	82.0	18.0	100.0

[a] Initial samples contained 1 μg Hg^{2+} ion in 500 ml water.

The reactivity of sulfur containing ligands towards preferential binding of mercury(II) by sulfur donor atoms has been reported in the literature [22,23]. Some preliminary experiments were carried out in order to investigate the quantitative retention of Hg^{2+} ions by the octadecyl membrane disk in the absence and presence of IIDE. It was found that, while the membrane disk itself does not show any tendency for the extraction of mercury ions, the membrane disk modified by ligand is capable to retain Hg^{2+} ions in the sample solutions quantitatively (the test solution contained 1 μg Hg^{2+} in 500 ml water).

3.1. Choice of eluent

In order to choose a proper eluent for the retained Hg^{2+} ions, after the extraction of 1 μg of mercury from 500 ml sample solution by the modified disks, the mercury ions were stripped with varying volumes of various stripping agents and the results are summarized in Table 1. From the data given in Table 1, of the different stripping agents used, two 8 ml portions of 0.5 M hydrobromic acid provides quantitative elution of mercury from the disk. It is interesting to note that, even utilizing an excess volume of 1 M nitric acid produced no loss of the retained Hg^{2+} ions on the modified membrane disks; thus, this solution can be used for the elution of some interfering ions, which may be co-extracted with Hg^{2+} ions by the membrane disk.

3.2. Effect of amount of ligand, flow rates and pH

The optimal amount of the ligand for the proper modification of the membrane disks was investigated. The results showed that the membrane disks modified with 2–20 mg of IIDE ligand are capable of retaining 1 μg of Hg^{2+} ions quantitatively. Thus, 10 mg of the ligand was used for further studies.

The effect of flow rate of the sample and stripping solution on the retention and recovery of mercury ion was investigated. It was found that, in the range of 10–80 ml min^{-1}, the reten-

tion of mercury by the membrane disk is not affected by the sample solution flow rate. Similar results for the extraction of organic and inorganic species by octadecyl silica membrane disks have already been reported [24,25]. On the other hand, quantitative stripping of the retained mercury ions from the disk was achieved at 1–15 ml min^{-1}, using two 8-ml portions of 0.5 M HBr. At higher flow rates, the recovery of mercury decreased in the first portion used for elution and larger volumes of 0.5 M HBr were needed.

The influence of the pH on the recovery of 1 μg Hg^{2+} from 500 ml solutions was studied in the pH range 3.0–7.0, the pH being adjusted by using either 0.1 M nitric acid or sodium hydroxide solutions. The recommended procedure was followed. In this range of pH, percent extraction of mercury was found to be independent of pH. It is interesting to note that a similar pH effect has already been reported for the extraction and separation of different metal ions with several ligands [9–11]. However, higher pH values (pH > 8) were not tested because of the possibility of hydrolysis of octadecyl silica in the membrane disks, resulting in decreased useful life-time of the disks [26].

3.3. Analytical performance

When solutions of 1 μg of mercury in 50, 100, 250, 500, 1000, 2000, 2500, 3000 ml sample solutions were passed through the modified disks, the Hg^{2+} ions were retained quantitatively. Thus, the break-through volume for the method should be greater than 3000 ml and, consequently, the concentration factor is greater than 150. The maximum capacity of the modified disks (containing 10 mg IIDE) was determined by passing 500 ml portions of an aqueous solution containing 1000 μg mercury followed by the CV-AAS determination of the retained metal ions. The maximum capacity of the disk was found to be 350 ± 30 μg of mercury. The stability and re-use of a modified disk was tested for its ability to perform the SPE of more than one sample. It was found that the use of the same disk modified with 10 mg IIDE for at least three times resulted in no change in the recovery of mercury. However, a single membrane disk can be used for the SPE of mercury ion from aqueous sample solutions over 15 times, providing its re-modification with the ligand after each use is undertaken. The limit of detection (LOD) of the proposed method for the determination of mercury was studied under optimal experimental conditions. The absolute LOD was found to be 5 ng, irrespective of the final volume of the sample (up to 3000 ml).

In order to investigate the selective separation and determination of Hg^{2+} ions from its binary mixtures with diverse metal ions, an aliquot of aqueous solution (500 ml) containing 1 μg Hg^{2+} and mg amounts of other cations was taken and the recommended procedures were followed. The results are summarized in Table 2. These data show that the mercury ions in the binary mixtures are retained completely by the modified membrane disk, even in the presence of 2–83 mg of different ions. As is obvious from Table 2, the maximum retention of foreign cations by the disk is <6% of the amounts taken. It is also worth mentioning that, in the cases of such interfering ions as Cd^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+} and Cr^{3+}, the increased amount of the diverse ions (in comparison with those reported in Table 2) resulted in some diminished recovery of Hg^{2+} ions. This is most probably due the increased competition of these cations, at such a relatively high levels, with Hg^{2+} for the ligand IIDE during the process of solid phase extraction. However, quantitative separation of foreign cations from Hg^{2+} ion can be achieved by washing the disks with 20 ml of 1 M HNO_3 solution before elution of the retained Hg^{2+} ions by HBr.

In order to assess the applicability of the proposed method to real samples with different matrices containing varying amounts of a variety of diverse ions, it was applied to the separation and recovery of 1 μg of Hg^{2+} ions from 500 ml solutions of a synthetic sample as well as three different natural water samples (i.e. rain, tap and river waters). The

Table 2
Separation of mercury from binary mixtures[a]

Divers ion	Amount taken (mg)	Found (%)[b]	Recovery of Hg^{2+} ion (%)
Ni^{2+}	23	1.0 (0.4)	100.8 (0.7)[c]
Pb^{2+}	17.0	5.3 (1.3)	97.5 (2.3)
Cd^{2+}	3.0	5.1 (0.7)	103.2 (2.1)
Zn^{2+}	25.0	3.1 (1.1)	97.4 (1.3)
Co^{2+}	22.0	0.9 (0.6)	99.6 (0.6)
Fe^{2+}	2.0	2.10 (1.1)	99.3 (3.2)
Mn^{2+}	2.2	5.5 (1.5)	98.8 (1.0)
Mg^{2+}	83.4	0.6 (1.2)	99.4 (1.1)
Ca^{2+}	70.0	1.0 (0.6)	99.3 (2.3)
Na^{+}	74.6	1.7 (1.3)	100.2 (0.8)
Cr^{3+}	2.0	3.7 (0.9)	100.4 (2.5)
Cu^{2+}	7.7	2.6 (1.2)	97.4 (1.3)
K^{+}	75.0	1.6 (1.2)	100.3 (1.1)

[a] Initial samples contained 1 μg Hg^{2+} and different amounts of diverse ions in 500 ml water.
[b] Percentage of the retained diverse cations was reduced dramatically by washing the disks with 20 ml of 1 M HNO_3.
[c] Values in parentheses are R.S.D.s based on three replicate analyses.

Table 3
Determination of mercury(II) in five human hair samples

No.	Sex	Age	Mercury concentration ($\mu g\,g^{-1}$)
1	M	15	1.23 (0.21)[a]
2	M	31	2.12 (0.23)
3	M	34	1.20 (0.22)
4	M	54	1.41 (0.30)
5	F	45	0.95 (0.32)
6	F (dyed)	27	23.57 (0.91)

[a] Values in parentheses are R.S.D.s based on three replicate analyses.

Table 4
Recovery tests carried out on hair sample number 2

Amount of Hg^{2+} (μg) added to 400 mg sample		
Added	Found	Recovery (%)
0.0	0.85 (0.12)[a]	–
0.5	1.32 (0.14)	94
1.0	1.82 (0.21)	97
2.0	2.71 (0.13)	95

[a] Values in parentheses are R.S.D.s based on three replicate analyses.

synthetic sample contained Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} (27 mg of each), Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+} and Cd^{2+} (2 mg of each). The results show that, in all samples, the mercury recovery is almost quantitative (98.3% in the case of synthetic sample and from 98.8 to 102.0 in the case of natural water samples).

The proposed method was also applied to the determination of Hg^{2+} ions in five human hair samples and the results are given in Table 3. The proposed method is suitable for the determination of mercury ions in human hair in the presence of different cations such as copper, lead, manganese and chromium.

The recovery of different added amounts of Hg^{2+} ions to the hair sample number 2 (see Table 3) was also carried out by the proposed method and the results are given in Table 4. The data given in Table 4 indicate that the recovery of the added mercury ions at all three levels tested is almost quantitative, although this may not reflect the real recovery of the endogenous mercury bound in the sample matrix.

4. Conclusion

The proposed SPE procedure based on octadecyl membrane disks modified with isopropyl 2-[(isopropoxycarbothioyl)disulfanyl]ethane thioate is a simple, rapid, highly selective and reproducible method for the separation, concentration and determination of mercury ion. The time taken for the separation, concentration and analysis of mercury in a 500 ml sample is about 30 min. The method can be successfully applied to the separation and determination of mercury in real samples.

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