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Solid-Phase Extraction and Spectrophotometric Determination of Trace Amounts of Mercury in Natural Samples

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Abstract. A sensitive and selective solid phase spectrophotometric method for the determination of trace amounts of inorganic mercury is described. Hg^{2+} was sorbed on a silica gel-packed column as an Hg^{2+}–*N*,*N*′-bis(2-mercaptophenyl)ethanediamide (H_2L) complex. The Hg^{2+} complex was eluted from the column using 7 mL of acetone. Various parameters including pH, column flow rate, and ligand concentration were optimized. The complex was found to obey Beer's law from 2.3 to 73.7 $\mu g\,mL^{-1}$ within the optimum range when the preconcentration factor was two. The effective molar absorption coefficient at 523 nm was $1.17 \times 10^3\,L\,mol^{-1}\,cm^{-1}$ at 523 nm. The concentration limits in Beer's law dropped from 0.09 to 2.95 $\mu g\,mL^{-1}$ within the optimum range when the preconcentration factor was 50. The relative standard deviation at a concentration level of 5 $\mu g\,mL^{-1}$ Hg^{2+} (9 repetitive determinations) was 1.6%. The detection limits are 0.34 $\mu g\,mL^{-1}$ and 0.015 $\mu g\,mL^{-1}$ when the preconcentration factors are 2 and 50, respectively. The method has been used for routine determination of trace levels of Hg^{2+} in natural waters. The potential application of this method for the removal of Hg^{2+} from natural samples (sea water and lake water) spiked with 100 $ng\,mL^{-1}$ of Hg^{2+} was studied. In order to validate the proposed method, LGC 6156 (harbour sediment – extractable metals) was analysed by this method. The results proved that excellent extraction of Hg^{2+} from both natural water samples was obtained by solid phase extraction using *N*,*N*′-bis(2-mercaptophenyl) ethanediamide.

Key words: Mercury; *N*,*N*′-bis(2-mercaptophenyl)ethanediamide; solid phase extraction.

The potential consequences of mercury bioaccumulation in aquatic eco-systems have been of great concern since the Minamata incident. Mercury is a potential environmental toxicant because of the strong affinity of inorganic (InHg) and organomercurial (OrHg) compounds for thiol groups, its tendency to form covalent bonds with organic molecules, the high stability of the Hg–C bond due to a low affinity for oxygen, and its strong tendency to maximize bonding to two ligands in a linear stereochemical arrangement [1]. In recent years, particular attention has been devoted to the presence of mercury species in natural waters which is recognized as an area of major environmental concern. The most dangereous form is methyl mercury, a potent neurotoxicant which concentrates in the blood and has an immediate and permanent effect on the brain and central nervous system. Inorganic mercury can be converted to the more dangerous methyl mercury by aquatic organisms. So there is a continuing need for the determination of mercury in natural waters. To effectively estimate the hazards involved, the variation in toxicity, transport and bioavailability,

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which are dependent on the chemical species of mercury, must be taken into account. A serious problem encountered in the determination of mercury is that the target species are usually present in low concentration. So the use of a preconcentration step is mandatory particularly if speciation analysis is to be carried out [2]. For mercury in natural waters, the main species to be identified and determined are inorganic mercury (Hg^{2+}) and methylmercury (CH_3Hg^+). It is important also to note that in natural waters methylmercury levels are usually much lower than those of inorganic mercury. Recent reports estimate the total mercury concentration in natural waters to range from 0.2 to 100 ng L^{-1}, while methylmercury levels are much lower (ca. 0.05 ng L^{-1}) [3].

Liquid–liquid extraction and separation of mercury in the presence of co-extractant ligands, such as aza-crown ethers dithizone, diethyldithiocarbamate, etc., has attracted considerable attention [4]. However, the use of classical extraction methods for this purpose is usually time-consuming, labor-intensive and requires large amounts of high purity solvents for extraction. Column solid-phase extraction has some advantages over liquid–liquid extraction: the possibility of combining it with UV-Vis spectrophotometry, AAS, as well as with ICP-AES, allows preconcentration from a larger sample volume, establishing higher preconcentration factors, simple storage and transportation of the pretreated samples [5–8]. Column preconcentration using chelating resins such as Chelex-100 [9, 10], quinolin-8-ol immobilized on porous glass [11], or silica [12] and poly(dithiocarbamate) [13], 1,5-bis[(2-pyridyl)-3-sulphophenyl methylene] thiocarbonohydrazide on anion-exchange resin [14] have been used for the enrichment of natural waters and biological materials. Chelating resins have found widespread applications in the enrichment of metal ions from various sources. In such resins, the chelating groups are responsible for metal ion enrichment, whereas the polymer backbone makes them more efficient by offering a large surface area. Nitrogen, oxygen and sulphur are the usual functional group atoms responsible for chelate formation. It has been reported that sulphur-containing ligands show greater affinity for heavy metals than oxygen and nitrogen [15].

The most widely used preconcentration methods are coprecipitation [16], ion exchange [17], solvent extraction [18, 19], and solid phase extraction [20–22]. Solid phase extraction (SPE) is an attractive technique that reduces solvent usage and exposure, disposal costs, and extraction time for sample preparation [23]. The basic principle behind SPE involves passing the water sample through a cartridge or a tube containing an adsorbent that retains analytes. After the sample has been passed through the tube or cardridge, the analytes are eluated from the adsorbent using a suitable solvent [24]. Synthesis of *N*,*N*′-bis(2-mercaptophenyl) ethanediamide (H_2L) as a new extractant was introduced by Kara for the spectrophotometric determination using solvent extraction. Then the reagent was used for the preconcentration and determination of Hg^{2+} at ppb levels in different water samples [25]. This study describes a procedure for the determination of inorganic mercury in natural water samples using the solid phase extraction technique. Several significant advantages should be obtained, including simple operation, freedom from interferences, excellent detection and avoiding the use of harmful organic solvents.

Experimental

Reagents and Apparatus

Absorbance measurements were made using a Cary 1-E UV-vis Spectrometer with 1.0 cm quartz cells. A pH meter (Nel pH 890) was also used. The peristaltic pump was a Watson Marlow 323 SD peristaltic pump. The micro column was a glass tube (0.7–10 cm Aldrich C 3669) packed with silica gel (1 g). Transport lines were made using 1.25 mm i.d. tygon tubing. All reagents were of analytical reagent grade (Merck) and deionized water was used throughout. A stock solution of 1000 μg mL^{-1} Hg^{2+} was prepared by placing appropriate amounts of analytical reagent grade $Hg(NO_3)_2$ in 2% (v/v) nitric acid solution. Working solutions were prepared immediately before use. Adjustment of pH was made with buffers (acetic and boric acids and their potassium salts). All glassware used was washed with 10% nitric acid for one day and rinsed with deionized water before use. The silica gel 60 for column chromatography used was 70–230 mesh and 0.063–0.20 mm particle size (Merck).

Synthesis of the Chelating Agent

H_2L (shown in Fig. 1) was prepared by refluxing 2 equivalents of 2-aminothiophenol (40 mmol) with 1 equivalent (20 mmol) of

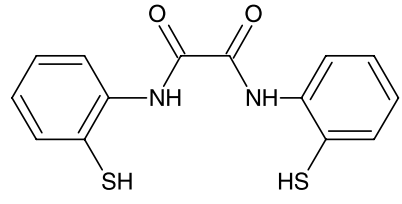

Fig. 1. Structure of *N*,*N*′-bis(2-mercaptophenyl)ethanediamide (H_2L)

diethyloxalate in 100 mL of ethanol for 4 h. The solution turned bright yellow, and on cooling the yellow amide solid appeared. The EtOH was removed by rotary evaporation, and the solid recrystallized from hot EtOH. The structure of the compound was confirmed by FT-IR and NMR spectrometry.

Preparation of the Column and Working Procedure

1 g of the silica gel was slurried in a glass column (0.7 cm × 10 cm) and thoroughly washed with doubly distilled water and then conditioned with pH 5.0 acetate buffer. The minicolumn was then connected to a peristaltic pump with PTFE tubing to form a preconcentration system. A solution containing certain amounts of Hg^{2+} was complexed with 0.5 mL of 10^{-2} M H_2L at the desired pH. Precipitation of mercury complexes in water media was prevented by adding 1 mL of 6% Triton X-100. The sorption of Hg^{2+} was studied at different flow rates, the adsorbed mercury complex on silica gel was eluted with 5 mL of acetone at different flow rates, and the concentration of Hg^{2+} in eluted solution was measured by UV-Vis spectrophotometry at 523 nm.

Results and Discussion

Selection of Sorbent

Firstly, C-18 silica gel was tried for the retention of mercury complexes. This revealed that the ligand (H_2L) completely adsorbed on the C18 sorbent, but the mercury complex formed in the solution was not retained. Then the use of silica gel was tried, and the result showed that silica gel completely adsorbed the Hg^{2+} complex. Therefore, silica gel was selected as sorbent in our studies.

Hg^{2+}–H_2L Colour System for Spectrophotometric Determination of Hg^{2+}

The absorption spectra of the ligand (H_2L) and the Hg^{2+}–H_2L complex are shown in Fig. 2. As seen, the spectra of the Hg^{2+}–H_2L complex have three maximums, two of which overlap with the maximum of the ligand at 364 and 264 nm. The other maximum appears at 523 nm where the ligand has no absorbance. Thus, the wavelength of 523 nm was used in all subsequent absorbance measurements.

Optimization of Experimental Parameters

The effect of time on complex formation between Hg^{2+} and ligand at pH 5.0 was studied at 523 nm. In all cases, equilibrium was attained in less than 6.5 min (Fig. 3). The waiting time required for full complexation was therefore selected to be 10 min.

Accurate results require careful control of pH values, since complexation efficiency is largely dependent on pH. In order to optimize the sorption conditions for the retention of the mercury complex on silica gel, the mercury complex signal was monitored by measuring it with UV-Vis spectrophotometry at 523 nm while changing the pH of the solution that passes through the minicolumn packed with silica gel. The absorbance values of eluated mercury complex at different pH values are given in Fig. 4. The maximum absorbance value of the metal complex showed at pH 5.0. Therefore this pH was selected as optimum pH for the system.

The effect of ligand concentration was studied by varying the volume of ligand between 0.5 and 3 mL of 10^{-2} M H_2L in 10 mL of 10 μg mL^{-1} Hg. When 1 mL or more of the ligand solution was added, the absorbance

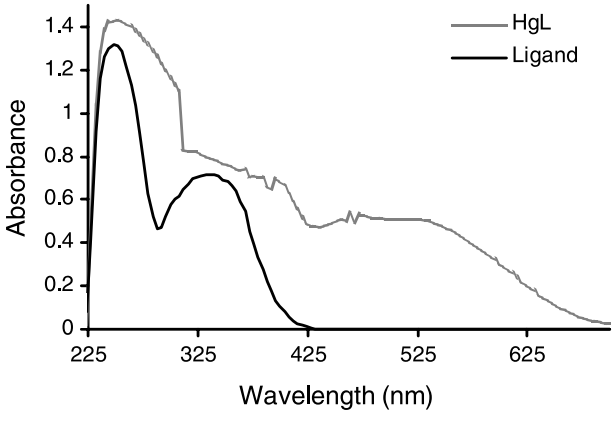

Fig. 2. Absorption spectra of the ligand (H_2L) and Hg^{2+} complex

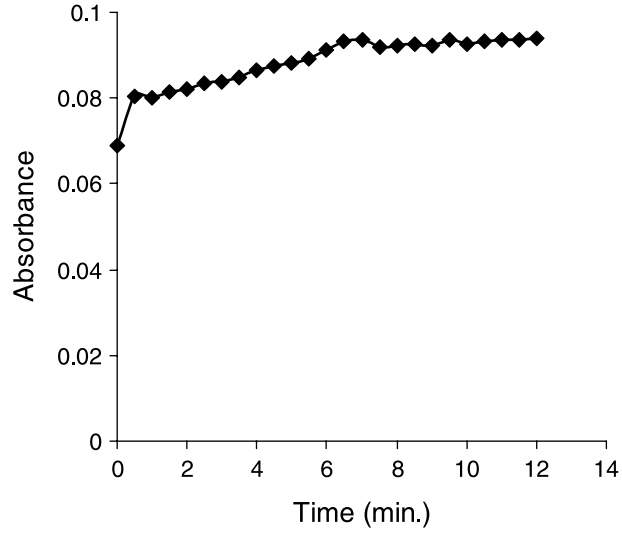

Fig. 3. Effect of complexation time

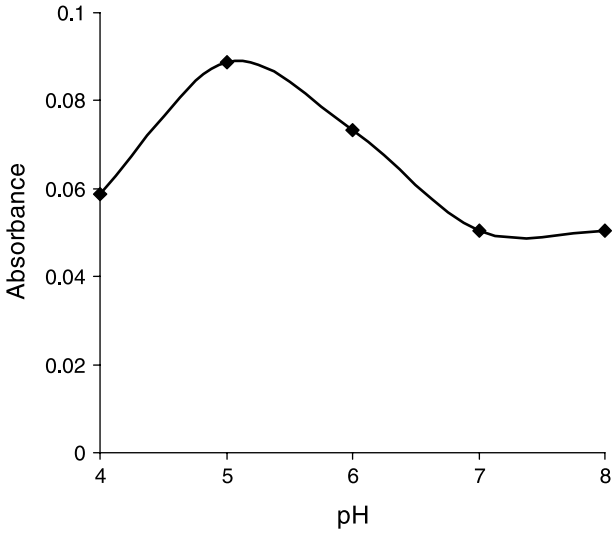

Fig. 4. Effect of pH on proposed method

value gradually decreased, which is probably due to competitive adsorption of the free ligand. Then 0.5 mL of 10^{-2} M ligand was selected as optimum amount of ligand. High sample loading flow rates are important for efficient preconcentration and for loading high volumes of sample within a very short time. No degradation of sorption efficiency was observed up to a loading flow rate of 20.0 mL min^{-1}. An optimum flow rate of 20 mL min^{-1} was used in all subsequent experiments. However, the flow rate of the eluent very much affects the absorbance signal of the complex. After a flow rate of 2 mL min^{-1} of eluent, the absorbance signal gradually decreases. Therefore, an elution flow rate of 2 mL min^{-1} was selected as optimum eluent flow rate.

Effect of Foreign Ions

The effects of foreign ions on the sorption of Hg^{2+} complex on a silica gel packed column were studied. A 10 mL solution containing 5 mg L^{-1} Hg^{2+} and various amounts of foreign ions was treated as described in the procedure. The results are given in Table 1. The presence of macro-amounts of foreign metal ions and some anions (SCN^-, CO_3^{2-}, tartarate, citrate and PO_4^{3-}) did not interfere with the absorbance measurement of the complex of Hg^{2+} or with the sorption of Hg^{2+} complex when present in 1000-fold excess. Interference from a number of anions (Br^-, Cl^-, I^- and NO_2^-) was observed when present in 100-fold excess. This concentration is much higher than that expected for natural waters, so no interference from foreign ions in real sample analysis is to be expected.

Table 1. Effect of foreign ions on the determination 10.00 mg L^{-1} of Hg^{2+}

Ion	Ratio ([Ion]/[Hg])	Found	% Error
Pb^{2+}	1000	9.96	−0.4
Zn^{2+}	1000	9.83	−1.7
Ca^{2+}	1000	10.02	0.2
Cd^{2+}	1000	9.99	−0.1
Ba^{2+}	1000	10.39	3.90
Cu^{2+}	100	9.38	−6.2
Ni^{2+}	100	9.54	−4.6
Co^{2+}	1000	9.66	−3.4
Al^{3+}	1000	9.72	−2.8
Cr^{3+}	1000	9.86	−1.4
Mn^{2+}	1000	9.40	6
Mg^{2+}	1000	10.46	4.6
NH_4^+	1000	10.20	2
SCN^-	1000	10.21	2.1
CO_3^{2-}	1000	10.06	0.6
Cl^-	100	10.22	2.2
Tartarate	1000	10.83	8.3
Citrate	1000	10.92	9.2
PO_4^{-3}	1000	9.76	−2.4
I^-	100	10.32	3.2
Br^-	100	11.20	11.2
NO_2^-	100	10.99	9.9

Evaluation of System Performance

The overall time required for the preconcentration of 100 mL of solution (5 min, at a flow rate of 20 mL min^{-1}); washing (0.25 min, at a flow rate of 20 mL min^{-1}); eluting (2.5 min, at a flow rate of 2.5 mL min^{-1}) and conditioning with buffer solution (0.25 min, at a flow rate of 20 mL min^{-1}) was approximately 8.0 min. A total enrichment factor of approximately 200 for a sample volume of 1000 mL was obtained with the proposed preconcentration system in 53 min of the overall time required for preconcentration.

The complex was found to obey Beer's law from 2.3 to 73.7 μg mL^{-1} within the optimum range when the preconcentration factor was 2. The effective molar absorption coefficient at 523 nm was 2.4×10^3 L mol^{-1} cm^{-1}. The accessible working range following Beer's law is changed to 0.09–2.95 μg mL^{-1} when the preconcentration factor is 50. The relative standard deviation at a concentration level of 5 μg mL^{-1} Hg^{2+} (9 repetitive determinations) was 1.6%. The detection limits are 0.34 μg mL^{-1} and 0.015 μg mL^{-1} when

Table 2. Comparable methods for solid phase extraction of traces of mercury

Reagent	pH	Linear range ($\mu g\,mL^{-1}$)	Detection limit ($ng\,L^{-1}$)	Application	Ref.
Dithizone loaded silica gel	–	–	20	tap water	26
6-mercaptopurine loaded polystryene DVB	5.5–6.0	up to 10	20	environmental samples	15
Methylthiosalicylate functionalized silica gel	–	5–1000	120/300	–	27
p-Sulfobenzylidenerhodanine	3.8	0.01–1.20	–	tobacco and tobacco additives	28
N,*N*′-bis(2-mercaptophenyl) ethanediamide	5.0	2.3–73.7 (PF 2) 0.09–2.95 (PF 50)	340 (PF 2) 15 (PF 50)	environmental samples	This study

the preconcentration factors are 2 and 50, respectively. This method can be applied to environmental samples highly contaminated with Hg^{2+}.

The method was compared with other solid phase extraction methods reported in the literature (Table 2). As can be seen from Table 2, the procedure developed is highly sensitive and easy to apply to the determination of Hg^{2+} concentration in aqueous solutions.

Measurement of Preconcentration Factor

For measurement of the preconcentration factor of the resin for Hg^{2+}, the proposed method was applied to 100, 250, 500, 750 and 1000 mL of aqueous solution containing certain amounts of Hg^{2+}. The Hg^{2+} in eluate was determined spectrophotometrically at 523 nm. The results are shown in Table 3. This method can be efficiently applied to large volumes of sample containing Hg^{2+}.

Application

In order to validate the methodology, the method proposed for mercury determination was applied to natural waters. Lake water samples containing mercury were collected from Sıgırcık station in Manyas Lake, Balıkesir, where effluents from various industries mix with lake water, and tap water samples were collected from Balıkesir. Sea water samples were collected from Marmara Bosphorous. The water samples were acidified using HNO_3 and were filtered through 0.45 μm pore size milipore membrane filters. The proposed method was applied to 500 mL of each sample after standard addition of certain amounts of Hg^{2+} and after adjusting

Table 3. Effect of preconcentration factor on solid phase extraction of mercury

Sample volume (mL)	Added Hg^{2+} ($mg\,L^{-1}$)	Preconcentration factor	Found Hg^{2+} ($mg\,L^{-1}$)	% Recovery
100	1	14.3	1.08 ± 0.07	108.3
250	0.4	35.7	0.40 ± 0.06	100
500	0.2	71.4	0.20 ± 0.015	100
750	0.1	107.1	$0.10 \pm 7.8 \times 10^{-4}$	100
1000	0.1	142.9	$0.098 \pm 4.9 \times 10^{-4}$	98.0

Table 4. Application of the preconcentration procedure to water samples

Sample	Added Hg^{2+} ($mg\,L^{-1}$)	Total Hg^{2+} found ($mg\,L^{-1}$)	Found sample concentration Hg^{2+}	Reference method ppb Hg^{2+}	Certified value ($mg\,kg^{-1}$)
Lake water	0.125	0.204	79.1 ± 4.5 ppb	80.4 ± 8.2[a]	–
Sea water	0.10	0.097 ± 0.0013	–	<0.5[b]	–
LGC 6156	–	–	$10.1 \pm 0.48\,mg\,kg^{-1}$	–	10.1 ± 1.6

[a] Result of CV-ICP-AES.
[b] Result of CV-AAS.

the pH to 5. After sample loading, the column was washed with 5 mL of distilled water, and the Hg^{2+} complex was eluated with 7 mL acetone. In order to validate the proposed method, LGC 6156 (harbour sediment – extractable metals) was analysed by this method. The results of the analysis are shown in Table 4.

Conclusions

In this study a detailed experimental analysis of mercury sorption on a silica gel packed column is presented. On the basis of the present study, the following conclusions can be drawn:

(i) the ligand is highly selective towards Hg^{2+} due to the presence of the soft basic S atom of the chelating reagent
(ii) the working pH range of this method is very suitable for natural waters
(iii) the presence of macro-amounts of foreign ions does not interfere with the sorption of Hg^{2+}
(iv) a preconcentration process has been proposed for the determination of Hg^{2+} in highly contaminated water samples
(v) the proposed procedure has some additional advantages in that the method features little interference from Cl^-, Br^-, I^- etc. in 100-fold excess, high precision and a low standard deviation
(vi) none of the cations interferes with the proposed method. This shows that the chelating ligand is very selective towards Hg^{2+} in the presence of other metal ions present in the system proposed. This is an important advantage of both the ligand and the proposed method.

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