Indian Journal of Chemistry
Vol. 55A, April 2016, pp. 423-428

Notes

Ultrasound assisted emulsification microextraction for selective determination of trace amount of mercury(II)

Leila Hajiaghababaei[a, *], Saeed Zandinejad[a],
Sana Berijani[b] & Saeed Suzangarzadeh[a]

[a]Department of Chemistry, Yadegar-e-Imam Khomeini (RAH) shahre-rey Branch, Islamic Azad University, Tehran, Iran
Email: lhajiaghababaei@yahoo.com
[b]Department of Applied Chemistry, Faculty of Science, Islamic Azad University, South Tehran Branch, Tehran, Iran

Received 8 April 2015; revised and accepted 16 March 2016

An environmental friendly sample pre-treatment method, ultrasound assisted emulsification microextraction, followed by furnace atomic absorption spectrometry is reported for the selective determination of mercury(II). Parameters that affect the extraction efficiency, such as the kind and volume of the extraction solvent, volume of chelating agent, sample *p*H, extraction time, temperature and addition of salt, are optimized. Under the optimum conditions for extraction recovery, 80 µL of tetrachloroethylene as extraction solvent and 1000 µL of [4-benzylidenamino- 3,4 dihydro-6-methyl 3-thioxo-1,2,4-triazin-5(2H)-one] as complexing agent give the best results. Under the optimum conditions, the calibration curve is linear in the range of 100-800 µg L^{-1}, relative standard deviation is 2.8% for five analysis of sample solution containing 0.3 mg L^{-1} Hg(II) with the limit of detection of 0.043 mg L^{-1}. The method has been applied successfully for assessing matrix effect by analysis of non-spiked and spiked real samples. The results demonstrate a successful robustness of the method for quantitative and selective determination of trace amount of mercury(II) in water and wastewater samples with relative recovery of 96-103%.

Keywords: Analytical chemistry, Ultrasound assisted emulsification microextraction, Graphite furnace atomic absorption spectrometry, Mercury

Mercury has specific characteristics that are largely employed for different industrial purposes, such as in thermometers, batteries and lamps. However, it is a toxic element found as contaminant in many different chemical forms. It can be easily inter-converted and transported through geochemical processes and pollute the environment. In the environment, mercury is absorbed by living organisms and is converted into its most toxic forms, CH_3Hg and C_2H_5Hg. When such contaminated food is consumed by humans, these toxic forms accumulate in many parts of the body, such as liver, muscles and brain[1]. Therefore, it is considered by the Environmental Protection Agency as a highly dangerous element. The World Health Organization (WHO) has set the allowed level of mercury in drinking water[2] at 1 g L^{-1}.

The development of reliable methods for the determination of mercury in environmental and biological materials is therefore quite important. Several analytical techniques have been used for the determination of mercury. The most commonly used technique is cold-vapor atomic absorption spectrometry[3-7]. It is a simple, fast, interference-free and sensitive technique. This method is accomplished by conversion of Hg(II) to volatile Hg(0) using a chemical reducing agent such as sodium tetrahydroborate(III) or stannous chloride. These chemical reducing agents have several drawbacks. Sodium tetrahydroborate(III) is an expensive reagent; it is prone to give high blanks due to contamination and is unstable in aqueous solution, and therefore, it has to be freshly prepared daily. It can also cause interference by reduction of several transition metals, while foam formation can be a problem with some samples. Causing bubbles, increased aerosol vapor in the gas–liquid separator and, poor precision are some of the other problems. Furthermore, production of hazardous laboratory wastes should not be underestimated when using chemical reducing agents[8]. Other techniques including electrothermal vaporization atomic absorption spectrometry[9-11], atomic fluorescence spectrometry[12,13], radiochemical neutron activation analysis[14,15], inductively coupled plasma mass spectrometry[16] and isotope mass spectrometry[17] have also been used. However, these methods are not very suitable for estimate of mercury, which is normally present in low concentrations in environmental samples[1]. Another option is to separate the analyte from the sample matrix. Therefore, a preconcentration and matrix elimination step prior to instrumental measurements is usually required in order to achieve accurate and reliable results.

Liquid-liquid extraction (LLE) requires large volumes of the sample and organic solvent and is time consuming, which make this technique tedious. Solid phase extraction (SPE), solid phase microextraction (SPME), and liquid-liquid microextraction (LLME) are other sample preparation techniques which could replace LLE[18-20]. He & Lee[21,22] introduced liquid phase microextraction (LPME) and single drop microextraction (SDME) as solvent minimized sample preparation procedures which have minimal exposure to organic solvent. However, for these methods some disadvantages such as breaking up at the organic solvent drop, air bubble formation and time-consuming have been reported[23]. Dispersive liquid-liquid microextraction (DLLME) was reported by Assadi *et al.*[24] It was applied for determination of organic compounds such as polyaromatic hydrocarbons[24], pesticides[25] and phthalate esters[26]. This method could also be applied to extraction of a wide range of different organic and inorganic compounds. DLLME is based on the dispersion of an organic solvent within the aqueous sample solution by a water-miscible organic solvent as a ternary component system. The main advantages of this technique are simplicity, rapidity, low volume of organic solvent, high enrichment factor, and compatibility with many instrumental analyses. However, this method suffers from the difficulty of automation and use of a disperser solvent that decreases the partition coefficient of analytes to extraction solvent[24-26]. Ultrasound assisted emulsification microextraction (USAEME) was introduced by Regueiro *et al.*[27] for extraction of contaminants and pesticides in water. Degassing, atomization, digestion and leaching are some of the applications of ultrasound energy in chemistry[28]. When a liquid is irradiated by ultrasound, and the pressure amplitude of the applied sound source reaches a certain minimum, cavitation occurs and this is known as the cavitation threshold. In an oil/water system, the process of emulsification is initiate when the cavitation threshold is attained. USAEME, coupled with other analytical techniques has been applied successfully for determination of different compounds[29-32]. Ultrasound energy accelerates the formation of fine droplets of the extraction solvent in a sample solution which causes better mass transfer and suitable emulsification. The major advantage of this technique over DLLEM is that emulsification is achieved without using a dispersive solvent. In the present study, USAEME followed by graphite furnace atomic absorption spectrometry (GFAAS) has been applied for preconcentration and selective extraction of mercury in water and wastewater samples. Herein, 4-benzylidenamino-3,4-dihydro-6-methyl-3-thioxo-1,2,4-triazin-5(2H)-one is used as a complexing agent for selective extraction of mercury ions. To the best our knowledge, this is the first application of this compound as complexing agent for mercury extraction in USAEME method.

Experimental

A stock standard solution of mercury at a concentration of 1000 µg mL^{-1} was prepared and working solutions were provided by serial dilutions of the stock solution with deionized water. 4-(Benzylidene amino)-3,4-dihydro-6-methyl-3-thioxo-1,2,4-triazin-5(2H)one was synthesized and purified as described elsewhere[33] and the chelating agent was prepared by dissolving the appropriate amount at concentration of 1000 µg mL^{-1} in ethanol. NaCl solution was prepared by dissolving the appropriate amount of NaCl in deionized water. Carbon tetrachloride, chloroform, tetrachloroethylene and chlorobenzene as extraction solvents and other salts were obtained from Merck (Darmstadt, Germany). The laboratory glassware was kept in 10% nitric acid prior to use.

The experiments were performed with a PG-900 (England PG Company) graphite furnace atomic absorption spectrometer. The conditions of the GFAAS were as follow: maximum wavelength for determination of mercury was 253.7 nm with 0.4 nm band width and 7 mA current. Argon with purity of 99.9% was used as carrier gas. For temperature programming in GFAAS, different programs were examined and the related calibration curves were obtained. The best program selected for this study is reported in Table 1. Varian UV-vis spectrophotometer (Cary-100) was used for investigating the selectivity of the chelating agent. A Ultrasonic bath (50-60 KHz and 750 W) with temperature control was used to assist the emulsification process of the microextraction technique. A *p*H meter (model PB-11), (Sartorius, Germany) was used for measuring *p*H of the prepared samples. A centrifuge (model 5702R, Eppedorf, Germany) was used to accelerate phase separation. For injection of organic phase drops in the sample solution and measuring the volume of the sedimented phase, 100 µL syringe was purchased from Hamilton (USA).

Table 1 –Temperature programming of GFAAS

Stage	Temp. (°C)	Ramp (s)	Hold (s)
Drying (1)	80	5	10
Drying (2)	120	10	15
Pyrolysis	200	10	15
Atomization	2000	0	3
Cleaning	2500	1	2

For the ultrasound assisted emulsification microextraction, aliquot of sample solution (5.0 mL) containing 300 µg L^{-1} of mercury was poured in a 10 mL screw cap glass tube with a conical bottom. Then, 1 mL of the chelating agent was added and 80 µL tetrachloroethylene (extraction solvent) was injected into the sample solution using a syringe while keeping the test tube in the ultrasonic bath. As a result, oil-in-water (O/W) emulsions of tetrachloroethylene (dispersed phase) in water (continuous phase) were formed. Disruption of the emulsion was carried out by centrifugation of emulsion at 4000 rpm for 3 min, which resulted in the organic phase sedimenting at the bottom of the conical tube. Finally, 10 µL of the sedimented phase (70 µL) was transferred directly to the graphite furnace for determination of mercury(II) without any dilution.

Results and discussion

In this study, the applicability of USAEME with graphite furnace atomic absorption spectrometry was explored as a simple and fast method for the preconcentration, extraction and determination of mercury(II) in environmental water samples by introducing a new and selective chelating agent, (4-benzylidenamino- 3,4 dihydro-6-methyl 3-thioxo-1,2,4-triazin-5(2H)-one). The selectivity of the chelating agent for mercury was investigated by UV-vis spectrophotometery. Equal amounts of various ions and varying amounts of mercury(II) were added to the chelating agent solutions individually. The UV-vis spectra were recorded (Supplementary data, Fig. S1) and changes in absorption were recorded. The spectra show that the chelating agent has a strong affinity for complexing with mercury(II) and hence can transfer the mercury ions to the solvent effectively.

The variables affecting the extraction recovery were studied and optimized. In the selected conditions, extraction efficiency or recovery and enrichment factor were calculated as follows[24,25]:

$$ER = \frac{C_p V_p}{C_i V_i} \times 100$$

$$EF = \frac{C_p}{C_i}$$

where ER and EF are extraction recovery and enrichment factor and C_p and C_i are mercury concentrations after preconcentration found by GF-AAS and initial concentration, respectively. V_p is the volume of organic acceptor phase after preconcentration and V_i is the volume of aqueous initial solution.

Selection of a suitable extraction solvent is critical to achieve an efficient USAEME procedure. The desired characteristics for appropriate extraction solvent are: low water solubility, high extraction capacity for the target analyte, form cation of a stable emulsion system under ultrasound energy, and compatibility with instrumental analysis system. Based on these considerations different solvents, $CHCl_3$, CCl_4, C_2Cl_4, C_6H_5Cl, were studied. Preliminary experiments were performed by using 100 µL of each solvent. The stable emulsion solution was obtained with each of these solvents except $CHCl_3$ which showed high solubility in water, and the experiments were repeated three times. Among the tested solvents, tetrachloroethylene(C_2Cl_4) gave almost quantitative results with formation of fine droplets of organic solvent in sample solution. Also, the distribution coefficient of the analyte into C_2Cl_4 was high. Hence in further experiments $C_2Cl_4$was used as extraction solvent.

Different volumes of the extraction solvent, ranging from 20–100 µL, were examined with the same USAEME procedures. The experiments showed that, when the volume of the extraction solvent increased, the recovery increased until 80 µL and then remained almost constant up to 100 µL, which indicates the high distribution coefficient of mercury-chelating agent in C_2Cl_4 and also quantitative analysis. Hence, 80 µL was selected as optimum volume of extraction solvent to achieve the best recovery and enrichment factor.

In order to establish an efficient UASEME procedure, *p*H of initial aqueous solution should be adjusted since the *p*H value of sample solution determines the existing state of the analyte and thus influences the extraction efficiency, especially for acidic or basic analytes and complex formation. As shown by the experiments, by increasing *p*H from 2 to 5, the extraction efficiency increased, however at *p*H>5, the efficiency decreased significantly (Supplementary data, Fig. S2). This is related to the formation of hydroxides of mercury in basic medias. Since the *p*H of the initial sample solution was 5, no adjustment of *p*H was needed in further experiments.

Transfer of ionic metals to the solvent is possible by using an appropriate chelating agent to convert the ions to a non-polar compound for extraction into the extraction solvent. Another important parameter is the volume of chelating agent for converting the highest amount of Hg(II) into the organic solvent extractable compound. Herein the effect of the volume of the chelating agent was investigated in the range of 200-2000 µL. The results indicate that by increasing the volume of the ligand, the efficiency of extraction also increases. However, with volumes larger than 1000 µL, the extraction efficiency decreased, which is probably because of the competition between the chelating agent and mercury ions for extracting the extraction solvent. Therefore, 1000 µL of the chelating agent was used in further analyses.

The influence of ionic strength on the performance of USAEME was investigated with NaCl of varying concentrations (0-10% *w*/*v*) in the sample solution. The results show that increasing ionic strength had no particular impact on the extraction efficiency, however the volume of deposited phase increased due to the reduction in solubility of organic solvent in water. Overall, addition of salt for nonpolar compounds decreased the solubility of analyte and promoted mass transfer and extraction efficiency. On the other hand, presence of salt in the sample solution increased density and viscosity of the solution which can prevent formation of fine droplets of organic phase. It is noteworthy that in the present study, even at high and variable levels of ionic strength, the responses were reproducible.

Extraction time is the time interval between the addition of extraction solvent and the end of sonication, prior to centrifugation. Time can affect emulsification and mass transfer process, and hence requires to be investigated to achieve the best response in minimum time. In the present study, extraction time was investigated in the range at 0–20 min. The results show that the extraction efficiency remains almost constant up to 20 min, indicates that the speed of extraction is high and independent of time. Hence, for further analysis, centrifuging was carried out immediately after addition of the extraction solvent.

Temperature can have an effect on the USAEME procedure, and consequently on the distribution coefficient and mass transfer of the analyte. To investigate the effect of temperature on the microextraction process, temperatures varying from 25 to 60 °C were examined. The results show that at temperatures higher than 25 °C, the extraction efficiency decreased. The reason is related to the increase in solubility of extraction solvent in aqueous solution in higher temperature. This prevents mass transfer and detracts extraction efficiencies. In the present study, the very large contact surface between organic solvent and the aqueous sample provides fast and efficient mass transfer of analyte. Therefore, all of the analyses were performed at room temperature.

The effect of some common coexisting ions in natural water samples were investigated on the extraction recovery of mercury. Various metal ions were added individually to standard solutions containing 300 µg L^{-1} of Hg(II). The tolerance limit was considered if it resulted in a ±5% variation in extraction efficiency of Hg(II). As can be seen in Table 2, the examined ions did not interfere significantly with the extraction and determination of mercury(II). The proposed method shows high tolerance limit for coexisting ions and the extraction recovery of Hg(II) was almost quantitative and selective in the presence of foreign ions and therefore may be useful for the analysis of Hg(II) in real samples.

The analytical performance of proposed method was validated under optimum conditions. Calibration graphs were constructed by using solutions of mercury(II) of known concentrations and the linear dynamic range (LDR) of the method was obtained over the range of 100–800 µg L^{-1} with the line equation as $Y = 0.6917X + 0.9475$ and regression coefficient $R^2 = 0.9972$.

The limit of detection (LOD) was determined by analysis of blank solution with proposed method under the optimal experimental conditions. The LOD obtained from $C_{\mathrm{LOD}}=\mathrm{K_b}S_b m^{-1}$ for a numerical factor

Table 2 – Tolerance limits for coexisting ions in preconcentration and separation of Hg(II)

Coexisting ion	[coexisting ion]/[Hg]	Rel. recovery (%)
Pb^{2+}	1000	97(±1.8)[a]
Ag^{+}	1000	96 (±2.4)
Zn^{2+}	1000	96(±1.9)
Cu^{2+}	500	96(±2.1)
Ni^{2+}	1000	98(±1.8)
Cr^{3+}	1000	96(±2.3)
Cd^{2+}	1000	96(±2.5)
Mn^{2+}	1000	97(±1.8)
Fe^{2+}	1000	96(±2.1)
Co^{2+}	1000	96(±2.3)
Ca^{2+}	10000	97(±1.8)
Na^{+}	10000	97(±2.4)

[a] RSD% based on three replicate analysis.

Table 3 – Determination of mercury(II) in standard solutions by USAEME-GFAAS and ICP-OES

Std samples	Sample 1 (mg L^{-1})	Sample 2 (mg L^{-1})
Cert. conc. of std solution	0.3	0.3
Found byUSAEME-GFAAS	0.31 (±0.02)[a]	0.28 (±0.03)
Found by ICP-OES	0.29 (±0.04)	0.30 (±0.03)

[a]RSD% based on three replicate analysis.

$K_b = 3$, was 0.043 mg L^{-1}, (S_b is standard deviation of blank solution and m is the slope of calibration curve). Precision, expressed as relative standard deviation (RSD%), was evaluated as 2.8% in terms of repeatability based on five replicate measurements of sample solution containing 0.3 mg L^{-1} Hg(II). The enrichment factor of the proposed method was 71.

The accuracy of the proposed method was evaluated by comparing of the results obtained by the proposed method and by inductively coupled plasma-optical emission spectrometry. The Hg(II) was analysed in some standard solutions with precise concentration. Also, the concentration of Hg(II)in the same standard solutions were determined by ICP-OES. As can be seen in Table 3, they are in satisfactory agreement.

The application of the method was examined by analyzing well water and laboratory wastewater of Islamic Azad University of Shahre Rey and well water of Tehran oil refinery. The samples were filtered using a 0.45-μm pore size membrane filter to remove suspended particulate matter. The solutions were spiked with varying amounts of mercury(II) since the certified waterid had very low concentration of the analyte. The results obtained by USAEME-GFAAS for spiked and non-spiked water and wastewater samples reveal that matrix has no adverse effect on efficiency of the method (Table 4).

A comparison of the proposed method with other reported liquid microextraction methods for mercury(II) determination is given in Table S1 (Supplementary data). The results clearly indicate that the extraction time of present method is superior to the previously reports such as single-drop microextraction (15 min)[34], cloud point extraction (15 min)[35] and dispersive liquid-liquid microextraction (35 min)[36]. In addition, this method has better precision in comparison with the above mentioned extraction methods, althouse, detection limit is higher than other methods. This is related to low detection limit of the inductively coupled plasma-optical emission spectrometry[35,36] and cold vapor atomic absorption spectrometry[37]. It must be mentioned that these instruments are expensive and not easily available. Therefore, the major advantages of the present extraction procedure are very short sample processing time, reduced channeling, requirement of small amount of harmful organic solvents, and, application in a variety of samples.

Table 4 – Determination of mercury in different water samples by USAEME-GFAAS

Real Sample	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD% (n=3)
Laboratory wastewater of Islamic Azad University of Shahre Rey	-	0.296	-	-
	0.1	0.399	103	2.9
	0.2	0489	97	2.1
Well water of Islamic Azad University of Shahre Rey	-	0.158	-	-
	0.1	0.254	96	1.8
	0.2	0.361	101	2.3
Well water of Tehran oil refinery	-	0.235	-	-
	0.1	0.331	96	1.9
	0.2	0.432	99	2.5

In the present study, USAEME coupled with GFAAS has been proposed for determination of Hg(II) in real samples. The method provides low detection limit, appropriate repeatability and good extraction recovery. In this method, the requirement of toxic organic solvent is very low with no effect on the performance, which is expected in other sample preparation techniques. Application of ultrasonic waves prompted an accelerated mass transfer and emulsification phenomenon. As a consequence, the reported method is a sensitive, efficient, rapid, easy and environmental friendly method, which can be used for quantitative analysis of low concentrations of mercury with satisfactory results. In addition, it can be applied successfully in water and wastewater.

Supplementary data

Supplementary data associated with this article i.e., Table S1 is available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_55A(04) 423-428_SupplData.pdf.

References

1 Saint'Pierre T D, Chavez Rocha R C & Duyck C B, *Microchem J*, 109 (2013) 41.

2 Bagheri H & Naderi M, *J Hazard Mater*, 165 (2009) 353.

3 Garcia I L, Cortez J A & Cordoba M H, *Atomic Spectros*, 14 (1993) 144.

4 Nguyen T H, Boman J, Leermakers M & Baeyens W, *J Anal Chem*, 360 (1998) 199.

5 Bettinelli M, Spezia S & Roberti S, *Atomic Spectros*, 20 (1999) 13.
6 Horvat M, Lupsina V & Pihlar B, *Anal Chim Acta*, 243 (1991) 71.
7 Pinkney A E, Logan D T & Wilson H T, *Arch Environ Contam Toxicol*, 33 (1997) 222.
8 Lavilla I & Bendicho C, *Spectrochim Acta B*, 62 (2007) 69.
9 Bagheri H & Naderi M, *J Hazard Mater*, 165 (2009) 353.
10 Araujo R G O, Vignola F, Castilho I N B, Borges D L G, Welz B, Goreti M, Vale R, Smichowski P, Ferreira S L C & Becker-Ross H, *Spectrochim Acta B,* 66 (2011) 378.
11 Torres D P, Dittert I M, Höhn H, Frescura V L A & Curtius A J, *Microchem J,* 96 (2010) 32.
12 Fernandez-Perez V, Garcia-Ayuso L E & de Castro M D L, *Analyst*, 125 (2000) 317.
13 Fernandez-Perez V, Jiminez-Carmona M M & de Castro M D L, *J Anal At Spectrom*, 14 (1999) 1761.
14 Norman B R & Becker D A, *J Radioanal Nucl Chem*, 245 (2000) 91.
15 Orvini E & Garlando P V, *Microchem J,* 46 (1992) 106.
16 Liao H C & Jiang S J, *Spectrochim Acta B,* 54 (1999) 1233.
17 Long S E & Robert Kelly W, *Anal Chem*, 74 (2002) 1477.
18 Junk G A & Richard J J, *J Anal Chem*, 60 (1988) 451.
19 Eisert R & Levsen K, *J Am Soc Mass Spectrom*, 6 (1995) 1119.
20 Cacho J, Ferreira V & Fernandez P, *Anal Chim Acta,* 264 (1992) 311.
21 He Y & Lee H K, *Anal Chem*, 69 (1997) 4634.
22 Wang Y, Kwok Y C, He Y & Lee H K, *Anal Chem*, 70 (1998) 4610.
23 Shen G & Lee H K, *Anal Chem*, 74 (2002) 648.
24 Rezaee M, Assadi Y, Milani Hosseini M R, Aghaee E, Ahmadi F & Berijani S, *J Chromatogr A*, 1116 (2006) 1.
25 Berijani S, Assadi Y, Anbia M, Milani Hosseini M R & Aghaee E, *J Chromatogr A*, 1123 (2006) 1.
26 Farahani H, Norouzi P, Dinarv R & Ganjali M R, *J Chromatogr A*, 1172 (2007) 105.
27 Regueiro J, Liompart M, Garcia-Jares C, Garcia-Monteagudo J C & Cela R, *J Chromatogr A*, 1190 (2008) 27.
28 Capote P F & Luque de Castro M D, *Anal Bioanal Chem*, 387 (2007) 249.
29 Ma J J, Du X, Zhang J W, Li J C & Wang L Z, *Talanta*, 80 (2009) 980.
30 Wu C, Liu N, Wu Q, Wang C & Wang Z, *Anal Chim Acta*, 679 (2010) 56.
31 Ozcan S, Tor A & Aydin M E, *Water Res*, 43 (2009) 4269.
32 Saleh A, Yamini Y, Faraji M, Rezaee M & Ghambarian M, *J Chromatogr A*, 1216, (2009) 6673.
33 Yadav S, Srivastava S, Pandey O P & Sengupta S K, *Synth React Inorg Met Org Chem*, 24 (1994) 925.
34 Sarafraz Yazdi A, Banihashemi S & Eshaghi Z, *Chromatographia*, 71 (2010) 1049.
35 Li Y & Hu B, *Spectrochim Acta Part B*, 62 (2007) 1153.
36 Sereshti H, Eskandarpour N, Samadi S & Aliakbarzadeh Gh, *Int J Environ Res*, 8 (2014) 1075.
37 Stanisz E, Werner J & Matusiewicz H, *Microchem J*, 110 (2013) 28.