اندازه‌گیری انتخابی مقادیر نانوگرم جیوه در نمونه‌های آب محیطی، ماهی و ادرار با استفاده از یک جاذب سنتزی جدید عامل‌دار شده و اسپکترومتری جذب اتمی بخار سرد بعد از بهینه‌سازی چند متغیره

شاهد حسن پور*۱، غلامرضا خیاطیان۲، امین زلالی۲

۱. بخش شیمی، دانشکده علوم، دانشگاه گیلان، رشت، ایران

۲. بخش شیمی، دانشکده علوم، دانشگاه کردستان، سنندج، ایران

Selective Determination of Nanogram Amounts of Mercury in Environmental Water, Fish and Urine Samples Using a New Synthetic Functionalized Sorbent and CVAAS after Multivariate Optimization

Shahed Hassanpoor[1]*, Gholamreza Khayatian[2], Amin Zolali[2]

1. Department of Chemistry, Faculty of Science, University of Guilan, Rasht, Iran
2. Department of Chemistry, Faculty of Science, University of Kurdistan, Sanandaj, Iran

چکیده

یک روش استخراج فاز جامد بر مبنای جاذب سیلیکا ژل عامل‌دارشده، برای پیش تغلیظ و اندازه‌گیری مقادیر ناچیز جیوه (II) توسط اسپکترومتری جذب اتمی بخار سرد ارائه گردید. پارامترهای مؤثر بر استخراج جیوه با استفاده از دو روش بهینه گردیدند: طراحی فاکتوریال جزئی و طراحی ترکیب مرکزی. فاکتورهای مؤثر بر بازیابی آنالیت به روش ناپیوسته مورد بررسی قرار گرفتند. بهترین شوینده برای واجذبی جیوه ۵ میلی لیتر تیواوره ۰/۱ مولار بود. مزاحمت تعداد زیادی از آنیون‌ها و کاتیون‌ها بررسی و روش انتخاب‌پذیری خیلی بالایی را برای تعیین جیوه در حضور سایر یون‌ها از خود نشان داد. تحت شرایط بهینه منحنی تجزیه‌ای در گستره ۱۶۰۰–۱۰ نانوگرم بر لیتر با حد تشخیص ۲/۳ نانوگرم بر لیتر تعیین گردید. روش بطور موفقیت‌آمیزی برای تعیین جیوه در نمونه‌های آب شیر، رودخانه و دریا، ادرار و ماهی بکار گرفته شد. نتایج بدست آمده منطبق بر نتایج بدست آمده توسط اسپکترومتری جذب اتمی الکتروال بودند. T–تست دلالت بر اعتبار و صحت روش پیشنهادی برای آنالیز جیوه در نمونه‌های حقیقی داشت.

واژه‌های کلیدی

تعیین جیوه؛ جاذب جدید؛ طراحی تجربی.

Abstract

A solid phase extraction method based on new functionalized silica gel sorbent for the preconcentration and determination of trace mercury (II) ions by cold vapor atomic absorption spectrometry (CV- AAS) was developed. The effective parameters on mercury extraction were optimized using two optimization methods: fractional factorial design and central composite design. Various factors affecting the recovery of the analyte were investigated using batch equilibrium technique. The best eluent for desorption of mercury was 5 mL of 0.1 M thiourea. Many anions and cations were examined in interference studies and the method shows very high selectivity for determination of mercury in presence of other species. Under the optimum conditions, the analytical curve was found to be linear in the range of 10-1600 ng L^{-1} with a detection limit of 2.3 ng L^{-1}. The method was successfully applied to determination of mercury in tap, river, sea water, urine and fish samples with good spike recoveries. The obtained results were in accordance with electrothermal atomic absorption spectrometric method. Student's t-test indicated the validity and accuracy of the proposed method for analysis of mercury in real samples.

Keywords

Mercury Determination; New Sorbent; Experimental Design.

1. INTRODUCTION

Mercury is regarded as one of the most toxic elements impacting on human and ecosystem health and is released into the environment from both anthropogenic and natural sources. As a result of population growth and urbanization,

*Corresponding Author: shahed_hassanpoor110@yahoo.com

more and more human activities have been contributing to significantly elevated mercury emission. More than 2500 tons of mercury is emitted annually from global anthropogenic sources [1]. Mercury is of considerable interest, as it is widely used in industry in the production of chemicals, pesticides, electrical apparatus, paints, amalgam tooth fillings, etc. and therefore, it is found throughout the ecosystem in trace amounts in air, water, soil and living organisms [2]. The ability of mercury to accumulate in biological tissues might eventually influence the entire food chain. Mercury can also be readily absorbed by the human body when inhaled by mucous membranes, damaging mainly the central nervous system [3]. In waters, inorganic mercury is converted by bacteria to methyl mercury, which is known to bio accumulate in the fish tissue. This process, additionally increases the danger of mercury exposure even at ultra-trace levels of concentration because organo-mercury species show a larger potential threat to human life and exhibit more toxic effects than inorganic mercury species [4]. Due to low concentrations of heavy metal ions in numerous samples and high levels of non-toxic components usually accompany analytes, a clean-up and preconcentration step is often required. Several preconcentration methods such as liquid-phase microextraction (LLME), cloud point extraction (CPE) and solid phase extraction (SPE) have been developed [5-7]. Solid phase extraction (SPE) is an effective technique that increases sensitivity, reduces analysis time, solvent usage and disposal costs for sample preparation. The most important disadvantages of SPE, are usually labor-intensive and require large amounts of sample volume for extraction [5].

Various solid-phase extraction materials including octadecyl silica membrane disks, activated carbon, agar powder, sulfur powder, magnetite nanoparticles, palladium wire and silica gel have been used for extraction and determination of mercury in various samples [4, 8-14]. Among all types of stationary phases used in solid-phase extraction (SPE), silica gel is the most common, due to its advantageous characteristics of thermal stability, no swelling and hydroxyl reactive groups [15]. But, most of these studies have been done with traditional one factor at a time (OFAT) method examining the effect of each factor individually holding all the other factors constant. It is well known that the OFAT is not a correct way for an optimization, or to study the effect of several factors in a measurement, since it does not take into account the interactions among factors. The OFAT only gives a local knowledge of the phenomenon and often requires a much larger experimental effort [16]. There is now increasing recognition that hereditary malpractice ought to be replaced by chemometrics based methods such as response surface methodology (RSM) and design of experiments (DOE) [17].

In the present study, a novel selective and sensitive sorbent for determination of mercury(II) in water, fish and urine samples, named 3,3'-Bis-(3-triethoxysilylpropyl)-2,2'-dithioxo-[5,5'] bithia-zolidinylidene-4,4'-dione- functionalized silica gel, was synthesized for the first time. The experimental variables in the extraction of mercury (II) were optimized using fractional factorial design (FFD) and central composite design (CCD). Mercury was determined using cold vapor atomic absorption spectrometry (CV-AAS) after reduction of mercury ions to elemental mercury by $SnCl_2$.

2. EXPERIMENTAL

2.1. Instrumentation
Determination of mercury was performed on a Varian Spectr AA 220 atomic absorption spectrometer (Australia) equipped with vapor generation accessory VGA-77 under the recommended conditions (wavelength 253.7 nm, bandwidth 0.5 nm and lamp current 4 mA). A standard Varian quartz absorption cell (17 cm × 1.5 cm) was placed directly on the burner equipped with cell tube holder and N_2 was used as the inert carrier gas and burner was turn off during of all experiments and measurements were carried out in the peak area mode. ETAAS measurements were also performed on the Varian Spectr AA 220 atomic absorption spectrometer (Australia) equipped with GTA-110 graphite tube atomizer and programmable sample dispenser (PSD). Pyrolytically coated graphite tubes were used for atomization and argon 99.99% purity was used as the inert gas. The pyrolysis temperature of 200°C and the atomization temperature of 1800°C were used. Measurements were carried out in the peak area mode using mercury hollow cathode lamps under the recommended conditions. All pH measurements were done using a Metrohm E-691 digital pH meter with a combined glass electrode. A model Labofuge 400 (Germany) centrifuge equipped with 100 ml glass tube and tube holder was used in extraction process. The stirring of the sample solutions was carried out by a magnetic stirrer (Rodwell, Monostir, England). FT-IR measurements were performed using KBr disk on an infrared spectrometer (Bruker-Vector 22).

2.2. Reagents and solutions
A 1000 mg L^{-1} standard solution of mercury (II) was prepared from Merck, (Darmstadt, Germany). $SnCl_2 \cdot 2H_2O$, carbon disulfide, dimethyl acetylene

dicarboxylate, 3-(triethoxysilyl) propan-1-amine, silica gel, triethylamine were procured from Merck (Darmstadt, Germany). The working standards were prepared immediately before use by serial dilution from a 1000 mg L^{-1} mercury stock solution in 0.05% nitric acid. A 25% w/v $SnCl_2$ in 20% HCl was prepared daily by dissolving appropriate amounts of $SnCl_2 \cdot 2H_2O$ in HCl on a hot plate and diluting with ultrapure water and was used as reducing agent. A 0.5 mol L^{-1} phosphate buffer adjusted to pH 1.70 was prepared by addition of an appropriate amount of sodium hydroxide (1.0 mol L^{-1}) to the phosphoric acid solution.

2.3. Synthesis of 3,3'-Bis-(3-triethoxysilylpropyl)-2,2'-dithioxo-[5,5']bithiazolidinylidene-4,4'-dione

The ligand was synthesized according to a procedure reported previously, [18] to a stirred solution of carbon disulfide (0.36 g, 4.8 mmol) and dimethyl acetylene dicarboxylate (0.28 g, 2 mmol) was added drop wised 3-(triethoxysilyl)propan-1-amine (0.88 g, 4 mmol) in 5 min. The reaction mixture was allowed to be stirred for 2 min. After reaction completed, the addition of EtOH to the reaction mixture generates product as orange crystals. M.p: 330 ºC decomposed. The IR and NMR data of the product were listed as follows:

IR (KBr, cm^{-1}); 3415, 1691(C=O), 1349 (C=S), 1158 (C-S); ^1H NMR (CDCl$_3$): δ ppm 0.64 (t, 4H, J = 7.3, 2CH$_2$), 1.15 (t, 6H, J = 7.3, 2CH$_3$), 1.58-1.67 (m, 4H, 2CH$_2$), 3.62 (t, 4H, J = 7.3, 2NCH$_2$), 4.09 (q, 4H, J = 7.2 Hz, 2OCH$_2$); ^{13}C NMR (CDCl$_3$) δ ppm 7.7 (2CH$_2$), 16.2 (2CH$_3$), 20.5 (2CH$_2$), 47.2 (2NCH$_2$), 57.1 (2OCH$_2$), 124.5 (2C), 167.2 (2C=O), 196.1 (2C=S).

2.4. Preparation of the sorbent

To a stirred mixture of silica gel (2 g) and ligand (0.2 g) in toluene (7 mL) was added triethylamine (0.2 mL). The reaction mixture was refluxed for 12 h, after this time the solvent was removed under reduced pressure. The residue was washed with 10 mL toluene that afford sorbent as brownish powder. Fig. 1 shows the process of preparation of sorbent. The IR data of the product were listed in the following:

Silica gel; IR (KBr, cm^{-1}); 3438 (OH), 1632, 1084, 800, 467 and sorbent; 3436 (OH), 1631, 1391 (C=S).

Fig. 2 shows the FT-IR spectra of ligand, silica gel and sorbent. The peak of C=O group in the sorbent is seen as a shoulder of the silica gel peak at 1631 cm-1. The peak appeared at 1391 is related to C=S band that confirms the reaction of the ligand and silica gel was successful to provide the new sorbent [19-21].
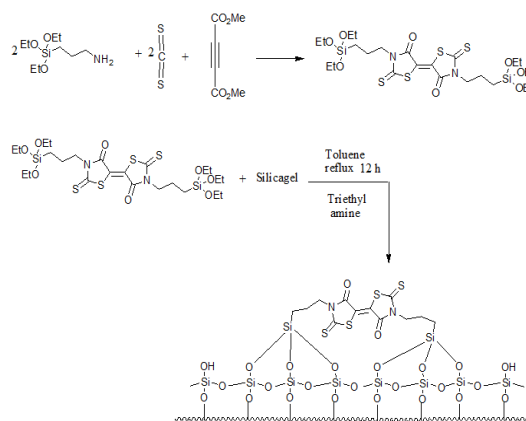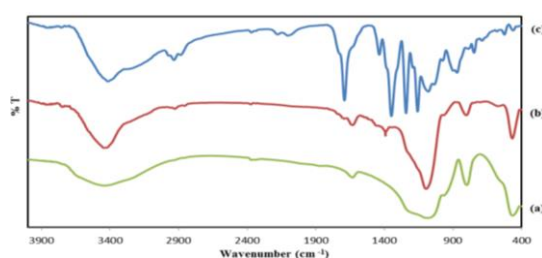

Fig. 1 Structure of ligand and sorbent.

Fig. 2 (a) FT-IR spectra of silicagel, (b) sorbent and (c) ligand.

2.5. Preparation of environmental water samples

The sea water from Caspian Sea (Rudsar, Gilan, IRAN), river water from Sepid Rood (Astaneh ashrafieh, Gilan, IRAN) and tap water (Sanandaj, Kurdistan, IRAN) were collected in 1.5 L polyethylene bottles. The samples were acidified to the pH <2 using 5 mL of concentrated HNO$_3$ and were filtered by a filter paper to remove suspended particular solids in the case of the sea and river water samples. Then, all samples were stored at about 4 °C in the refrigerator. In order to determine the total mercury, 10 mL of water sample was transferred to a round-bottom flask, 0.4 mL of H$_2$SO$_4$ (98%), 0.15 mL of HNO$_3$ (70%), 0.4 mL of K$_2$S$_2$O$_8$ (5%) and 0.75 mL of KMnO$_4$ (5%) were added and refluxed at 80 °C for 2 h. This solution was cooled, neutralized with sodium hydroxide and diluted to 100 mL in a volumetric flask and it was treated under recommended procedure. [4] For ET-AAS method, total mercury was determined by introduction of the samples to the GTA after filtration.

2.6. Preparation of fish samples

Three fish samples were collected from Caspian sea and its environment rivers, Caspian Vimba (Shalman river, Shalman, Gilan, IRAN), Caspian Grass Carp (Shalman river, Shalman, Gilan, IRAN) and Caspian Kutum (Caspian sea, Kiya Shahr sea port, Gilan, IRAN). For determining the total mercury in fish samples, 1.0 g of dried spinal muscle of fish was placed in a round-bottom flask

and 10 mL of HNO_3 (70%), 10 mL of H_2SO_4 (98%), 10 mL of $K_2S_2O_8$ (5%) and 8 mL of $KMnO_4$ (5%) were added to a round-bottom flask and the mixture was refluxed at ~250 °C for about 2 h. The resulting solution was then cooled to room temperature, neutralized and diluted to 1000 mL with ultrapure water. Then 100 mL of this solution was treated under the recommended procedure [4]. In the case of ET-AAS method, the final volume of digested solution was 100 mL and total mercury was determined by introduction of the samples to the GTA.

2.7. Preparation of urine samples
For determining the total mercury in urine samples, 10 mL urine sample, 0.5 mL of H_2SO_4 (98%), 0.2 mL of HNO_3 (70%), 0.5 mL of $K_2S_2O_8$ (5%) and 1 mL of $KMnO_4$ (5%) were added to a round-bottom flask and refluxed at ~80 °C for 2 h. This solution was cooled, neutralized with sodium hydroxide and then diluted to the mark in 100 mL volumetric flask [22]. In the case of ET-AAS method, the final volume of digested solution was 10 mL and total mercury was determined by introduction of the samples to the GTA.

2.8. General procedure
In a 100 mL centrifuge glass tube, 100 mL of 0.5 μg L^{-1} of mercury(II) solution or real sample, 5 mL phosphate buffer 0.5 M pH= 1.7 (concentration in solution is 0.024M) and 115 mg of sorbent were added. The tube was put on the stirrer for 16 min at speed of 100 rpm. Then, the mixture was centrifuged at 3500 rpm for 5 min, after phase separation, the clear solution was removed. For desorption of the adsorbed mercury ions, the sorbent were rinsed with 5 mL 0.1 mol L^{-1} thiourea solution for 10 min on the stirrer at speed of 100 rpm. Then, the mixture was centrifuged at 3500 rpm for 5 min and the solution was transferred in a 5 mL flask and diluted to mark if necessary. Finally, the extractant was analyzed for mercury determination by CV-AAS.

3. RESULT AND DISCUSSION
The eluent for mercury desorption was investigated in the first step in order to achieve high recovery and absorbance for mercury. The main factors affecting the recovery of mercury, such as amount of sorbent, pH, extraction time, salt addition, stirring rate and time, a design with two steps (screening and optimization) was used for the experimental conditions. For this purpose, MINITAB16.2.2 (Minitab Inc., LEAD Technologies, Inc.) statistical package was used

to generate the experimental matrix and to evaluate the results.

3.1. Effect of type and concentration of desorbing reagent
In order to choose a proper desorbing reagent for recovery of mercury (II) ions, 5 mL of various reagents were added to the sorbent after extraction of mercury from 100 mL 0.5 μg L-1 of mercury solution. In this study, all of the experimental conditions were in the central point. The results are shown in Table1. As can be seen from Table 1, among the different reagents, 5 mL thiourea 0.1 M provides highest recovery. Therefore, this solution was chosen as an eluent for further study.

Table 1. Effect of type and concentration of desorbing reagent on the recovery of from 100 mL 0.5 μg L^{-1} of mercury (II) solution. Conditions: all of the factors were in the central points (N = 3)

Eluent	Concentration (molL^{-1})			Recovery (%)		
HCl	0.1	0.5	1	11.9 ± 0.7 [a]	20.8 ± 1.2	23.8 ± 0.7
HNO_3	0.1	0.5	1	35.2 ± 1	50.8 ± 1.2	63 ± 2
EDTA	0.01	0.1	-	13.3 ± 0.7	40.6 ± 0.7	-
$Na_2S_2O_3$	0.1	0.5	-	41.4 ± 1.5	50.2 ± 2	56 ± 1.5
Thiourea	0.1	0.5	-	63.1 ± 1.2	63.2 ± 1.5	-
NaSCN	0.1	0.5	-	15.4 ± 1	25.6 ± 0.7	34 ± 1

[a] Mean ± standard deviation.

3.2. Experimental design
In this study, optimization involved the maximization of recovery which assumed to be a function of several independent variables. Many methods have been devised to achieve this purpose. Among these methods, most commonly utilized by chemists is the technique of experimental design. The design of experiments is a part of response surface modeling (RSM) methodology [23]. There are many possible applications of these techniques in chemistry. They may be used, for example, to optimize the yield of reaction, to decrease the level of impurity present in an end product, to improve a chromatographic separation or optimization of extraction. Indeed, response surface is a graph of a response as a function of one or more factors. [24] For an optimization for comparison traditional one factor at a time (OFAT) with response surface modeling, the change OFAT approach does not even reach the true optimum [25]. By application of RSM, it is possible to evaluate the interactions of possible influencing factors on treatment

efficiency with a limited number of planned experiments [26].

3.2.1. Fractional factorial design (FFD)

In the first step, the main factors affecting the extraction and recovery of mercury were determined. The main factors, their symbols and levels are shown in Table 2. As can be seen in Table 2, each variable was set at two possible levels, high and low, thus there are $2^7 = 128$ possible combination in full factorial design. This full factorial design is too many to carry out practice. For this reason, 2^{7-3} fractional factorial design were used in our experiments and these run in a random manner to minimize the effect of uncontrolled variables.

Table 2. Factors, their levels and symbols for fractional factorial design.

Factors	Symbol	Low (-1)	Central (0)	High (+1)
Amount of sorbent (mg)	A	20	60	100
pH	B	1	4	7
Extraction Time (min)	C	2	9	16
Salt (% W/V NaCl)	D	0	2.5	5
Speed of stirring (rpm)	E	100	550	1000
Time of desorption (min)	F	1	5.5	10
Speed of desorption stirring (rpm)	G	100	400	700

Factors: 7, Resolution with blocks: IV, Runs: 20, Replicates: 1, Fraction: 1/8, Blocks: 2, Center points (total): 4.

Because the run time was not short enough to perform experiments during one working day, they were divided into two blocks and the two center points were added for each block for investigation of error estimate and curvature in the response. The design matrix and the responses are shown in Table 3.

Table 3. Design matrix and the recovery for 27-3 fractional factorial design.

Run	Blocks	A	B	C	D	E	F	G	Recovery %
1	1	-1	+1	-1	+1	+1	-1	+1	21.9
2	1	0	0	0	0	0	0	0	62.1
3	1	+1	-1	+1	+1	-1	-1	+1	69.7
4	1	+1	-1	-1	+1	+1	+1	-1	36.2
5	1	0	0	0	0	0	0	0	60.5
6	1	+1	+1	-1	-1	-1	+1	+	31.8
7	1	-1	-1	-1	-1	-1	-1	-1	30.3
8	1	-1	-1	+1	-1	+1	+1	+1	33.9
9	1	+1	+1	+1	-1	+1	-1	-1	52.5
10	1	-1	+1	+1	+1	-1	+1	-1	15.4
11	2	-1	-1	+1	+1	+1	-1	-1	29.0
12	2	-1	+1	+1	-1	-1	-1	+1	11.8
13	2	+1	+1	+1	+1	+1	+1	+1	53.6
14	2	-1	-1	-1	+1	-1	+1	+1	29.1
15	2	0	0	0	0	0	0	0	61.2
16	2	+1	-1	+1	-1	-1	+1	-1	95.5
17	2	0	0	0	0	0	0	0	61.7
18	2	+1	+1	-1	+1	-1	-1	-1	31.4
19	2	+1	-1	-1	-1	+1	-1	+1	50.0
20	2	-1	+1	-1	-1	+1	+1	-1	21.0

The analysis of variance (ANOVA) calculated with Minitab software package was shown in Table 4. In this table, values of $p < 0.05$ indicate the model terms are significant. Therefore, the main effects of A, C, D and B is large and the two factor interactions AC, BD, AB, AD and AE also appear to be important. A normal plot of these effects also shows that these effects are only ones to influence the recovery of mercury (Fig.3). The normal plot shows that salt has a negative effect in the recovery of mercury. Hence, no salt was added in the subsequence experiments.

Table 4. Analysis of Variance (ANOVA) for fractional factorial design (coded units).

Source	Effect	Seq SS	DF	Adj MS	F value	P value
Blocks		43.51	1	43.51	11.65	0.042
Main Effects		5252.89	7	750.41	200.91	0.001
A	28.475	3243.30	1	3243.30	868.35	0.000
B	-16.725	1118.90	1	1118.90	299.57	0.000
C	13.650	745.29	1	745.29	199.54	0.001
D	-5.000	100.00	1	100.00	26.77	0.014
E	-2.050	16.81	1	16.81	4.50	0.124
F	2.425	23.52	1	23.52	6.30	0.087
G	-1.125	5.06	1	5.06	1.36	0.329
2-Way Interactions		1610.96	7	230.14	61.62	0.003
A× B	-3.675	54.02	1	54.02	14.46	0.032
A× C	16.700	1115.56	1	1115.56	298.68	0.000
A× D	-4.600	84.64	1	84.64	22.66	0.018
A× E	-6.850	187.69	1	187.69	50.25	0.006
A× F	0.825	2.72	1	2.72	0.73	0.456
A× G	-1.375	7.56	1	7.56	2.02	0.250
B× D	6.300	158.76	1	158.76	42.51	0.007
Curvature		1705.70	1	1705.70	456.68	0.000
Residual Error		3.74	3	3.74		
Lack of Fit		9.80	2	9.80	13.95	0.065
Pure Error		0.70	1	0.70		
Total		8624.27	19			

This phenomenon may be due to a tendency to adhere of the sorbent to the walls of the glass tube decreasing the recovery of mercury at very high rotation speeds. Desorption time has little positive effect (F in Table 4) so, a high level (10 min) was selected for the further experiments. With these results and screening, the three important factors in extraction and determination of mercury were amount of sorbent, pH and extraction time. Thus, for more accurate investigation, central composite design was used in the next step.
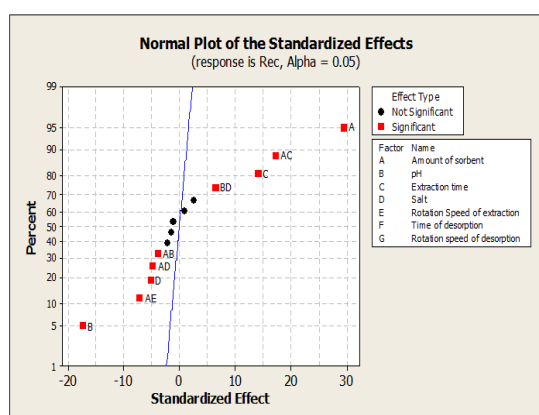

Fig.3 Normal plot obtained from the results of fractional factorial design.

3.2.2. Central composite design (CCD)

As can be easily seen in Table 4, the curvature in fractional factorial design model is significant. For this reason, a design is needed with more levels. Two-level designs can only lead to linear models of responses giving no information about maxima or any non-linear relationships. Central composite designs require 2^k factorial points (also called cube points) + 2k axial points (also called star points) + nc center points run of experiments. A central composite design overcomes such problems presented by factorial design and star design. This kind of design provides the same efficiency as a factorial design at three levels but with a major number of concentration levels [16, 25, 27].

The factors, their levels, symbols and design matrix for central composite designs are shown in Tables 5 and 6. The central composite design allows estimating the constant, linear terms, interactions between variables and the quadratic terms, according to the following model:

$R = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x_i^2$

For this design, the equation in coded value was

% Recovery = -61.4284 + 1.5376 A+ 15.5993 B + 5.0706 C -0.0081 A^2- 1.9239 B^2-0.1754 C^2-0.0549 AB + 0.0253 AC -0.2286 BC

As can be seen, in the recovery equation, pH is most significant in such a way that the pH and extraction time interaction and quadratic term of pH was also significant.

Table 5. Factors, their levels and symbols for central composite designs.

Factors	Symbol	Low (-1)	Central (0)	High (+1)
Amount of sorbent (mg)	A	50	75	100
pH	B	1.5	3.25	5
Extraction time (min)	C	5	12.5	20

Factors:3, Replicates: 1, Base runs: 20, Total runs:20, Base blocks:2, Total blocks:2, Two-level factorial: Full factorial, Cube points: 8, Center points in cube: 4, Axial points: 6, Center points in axial: 2, Alpha: 1.633.

Table 6. Design matrix and the recovery for central composite designs.

Run	Blocks	A	B	C	Recovery %
1	1	0	0	0	75.1
2	1	-1	+1	+1	45.6
3	1	+1	+1	+1	73
4	1	+1	-1	+1	97.3
5	1	+1	+1	-1	42
6	1	0	0	0	75.3
7	1	0	0	0	75.4
8	1	+1	-1	-1	54
9	1	0	0	0	76.3
10	1	-1	-1	-1	36
11	2	-1	+1	-1	33.3
12	2	-1	-1	+1	60
13	2	0	0	0	76.1
14	2	0	0	0	75.9
15	2	-1.633	0	0	43
16	2	+1.633	0	0	80
17	2	0	0	+1.633	71.3
18	2	0	0	-1.633	26.2
19	2	0	+1.633	0	48
20	2	0	-1.633	0	70.7

The analysis of variance (ANOVA) calculated with Minitab software package was shown in Table 7. In this table, regression value of model was significant and lack of fit of the model was not significant. From these results, the efficiency of the model was inferred.

Table 7. Analysis of Variance for central composite designs (coded units).

Source	Seq SS	DF	Adj MS	F value	P value
Blocks	1.41	1	1.41	4.10	0.074
Regression	7047.12	9	783.01	2278.13	0.000
Linear	4888.61	3	298.96	869.81	0.000
A	1728.72	1	412.84	1201.13	0.000
B	613.85	1	287.52	836.53	0.000
C	2546.05	1	584.93	1701.83	0.000
Square	1859.92	3	619.97	1803.78	0.000
A×A	217.08	1	341.56	993.75	0.000
B×B	357.14	1	458.44	1333.81	0.000
C×C	1285.70	1	1285.70	3740.66	0.000
Interaction	298.58	3	99.53	289.57	0.000
A× B	46.08	1	46.08	134.07	0.000
A× C	180.50	1	180.50	525.15	0.000
B× C	72.00	1	72.00	209.48	0.000
Residual Error	0.75	9	0.08		
Lack-of-Fit	2.23	5	0.45	2.05	0.253
Pure Error	0.87	4	0.22		
Total	7051.62	19			

3.3. Response surface and selection of optimum condition

The obtained regression model was used to calculate the three dimensional (3D) two factor response surface, when one of the variables is fixed at the central point and the other are allowed varying (Fig. 4). These figures show the existence of maximum in the recovery and interaction between the factors. Fig. 4.a shows that the recovery decreases ~30% at low amounts of sorbent. This decrease is due to lower adsorption capacity of adsorbent in the lower amounts. Fig. 4.a and Fig. 4.c show the great importance of pH in complex formation and extraction of mercury. The recovery increases by increasing the pH up to 1.5 and then decreases with pH beyond 2. The decrease in extraction of mercury (II) ions at higher pHs may be due to competition of hydroxyl ion with sorbent to react with analyte, while a decrease in lower pHs is due to protonation of sorbent at these pHs. Therefore a pH 1.7 was chosen for subsequent experiments and the pH was adjusted by addition of 5 mL of 0.5 M phosphate buffer with pH= 1.7 to the sample solution. As can be seen from Fig. 4.b and 4.c, the mercury extraction time is important and its maximum recovery is obtained in the extraction time of 21 min. Therefore, according to screening, optimization study and global solution of Minitab software response optimizer,115 mg of sorbent, pH=1.7, 21 min for extraction time, 0 % of salt and 100 rpm stirring rate for extraction, 10 min for time of desorption and 100 rpm stirring rate for desorption were selected as the optimum values for determination and extraction of mercury. The predicted response recovery was 101.03 %.
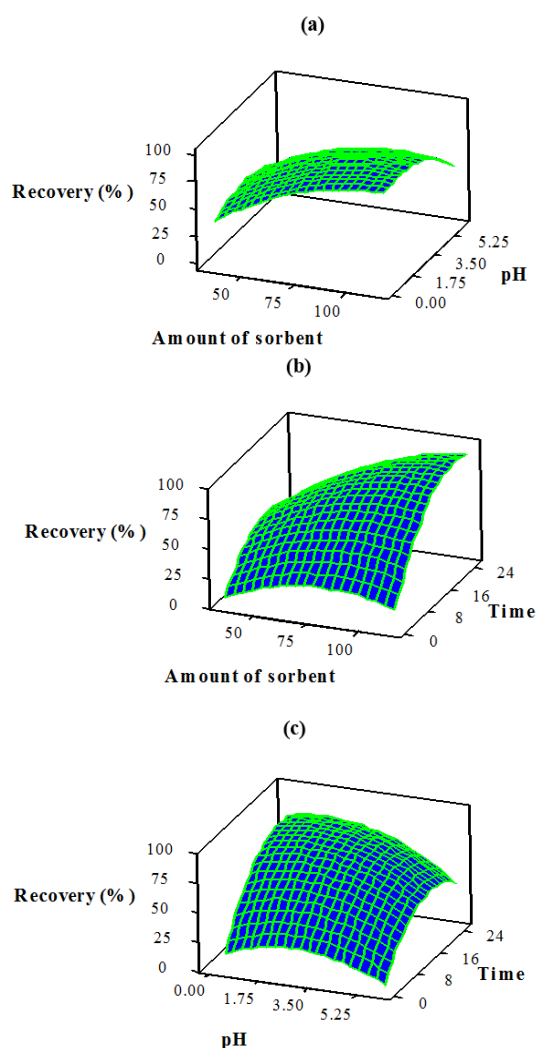
Fig. 4. The three dimensional (3D) central composite design plots for the effects of variables on recovery of mercury (II).

3.4. Effect of the sample volume and enrichment factor

In order to investigate the breakthrough volume, sample solutions of 25, 50, 100, 150, 200, 250, 300, 350, 375, 400 mL containing 0.05 μg of mercury (II) were studied according to the recommended procedure. For experiments using higher sample volumes than 100 mL, the extraction procedure was done in an appropriate beaker. After collection of the sorbent and desorption of the analyte, determination of mercury was done under the recommended procedure. It was found that mercury could be quantitatively recovered up to 350 mL of the sample volume. Thus, the enrichment factor of the method was calculated to be about 70.

3.5. Effect of diverse ions

The effects of common potentially interfering ions on the recovery of mercury (II) were also studied. In these experiments, 100 mL of solutions containing mercury (II) (500 ng L^{-1}) and various amounts of diverse ions were treated according to the recommended procedure under optimum condition. A given species was considered to interfere if it resulted in a ±5 % variation of the absorbance signal. As shown in Table 8, most of the cations, anions and organic species had no obvious influence on the determination of mercury under selected conditions. The result shows that, the method is highly selective in the presence of different species. This may be due to formation of more stable complexes of Hg (II) as a soft acid with sulfur atoms of the ligand as a soft base in the new synthetic sorbent compared to other metal ions studied [28].

Table 8. Effect of coexisting ions on the extraction of 500 ng L^{-1} mercury (II).

Coexisting ions	Tolerance limit (mg L^{-1})
Na$^+$, NO$_3^-$	2500
K$^+$, Cl$^-$	2000
Ba^{2+}, F$^-$, Acetate	1500
Mg^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Ca^{2+}, NH$_4^+$	800
CO$_3^{2-}$, Oxalate	500
Zn^{2+}, EDTA, SO$_4^{2-}$, Citrate	400
Cu^{2+}, Al^{3+}, Cr^{3+}, Urea	300
Pb^{2+}, Cd^{2+}, Bi^{3+}, I$^-$	200
Ag$^+$, Mn^{2+}	100
As^{3+}	30

3.6. Reusability and loading capacity of the sorbent

The reusability of the sorbent in several successive adsorption and desorption processes was studied. The obtained results showed that the sorbent could be reused up to 70 times without any considerable loss in its adsorption efficiency. The maximum loading capacity of the sorbent was obtained from the injection of 115 mg of sorbent to a 100 mL of 5.0 μg L^{-1} mercury (II) solution and the determination of mercury in eluent according to the recommended procedure. The loading capacity was 1.91μg g^{-1}.

3.7. Analytical figures of merit

The analytical characteristics of the proposed method, including linear range, limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD), correlation coefficient (R^2), and enrichment factor were obtained. Under the optimum experimental conditions, analytical curve was achieved by analyzing 100 mL of mercury (II) standard solution containing a known amount of target ion in the range of 5.0–2000 ng L^{-1}. The analytical curve was linear in the range of 10.0–1600 ng L^{-1} with a correlation coefficient (R^2) of 0.999. The regression equation was A = 1.310 C - 0.01, where A is the absorbance and C is the concentration of mercury (II) in ng mL^{-1}. The limit of detection (n = 10, LOD = 3σ$_{blank}$ / m) and limit of quantification (n=10, LOQ = 10 σ$_{blank}$/ m) where m is the slope of the analytical curve in accordance to IUPAC recommendation, were obtained 2.3 ng L^{-1} and 7.6 ng L^{-1} respectively. The RSD for ten replicate measurements of 500 ng L^{-1} of mercury (II) ions was 2% and the recovery of extraction calculated according to: (C$_e$ .V$_e$) / (C$_s$. V$_s$) × 100, where C$_e$ and C$_s$ are the concentrations of mercury in eluent and sample solution, V$_e$ and V$_s$ are the volumes of the eluent and the sample solution respectively, was about 98.5 %. The C$_e$ calculated with separate calibration curve with addition of known amount of mercury with different concentration in eluent matrix

3.8. Applications

The proposed method was successfully applied for determination of total mercury in different natural waters, urine and fish samples. The results along with the recovery for the spiked samples and ET-AAS method were given in Tables 9, 10. The accuracy of proposed method was evaluated by means of recovery experiments and analysis of samples with ET-AAS method. As can be seen, the added mercury was quantitatively recovered from all samples and the obtained results are in good agreement with ET-AAS values. A student's t- test indicated that there was no significant difference between SPE-CV-AAS and ET-AAS experimental results. These results indicate the

validity of the proposed methodology for analysis of mercury in real samples.

Separation and determination of mercury by developed method were compared with the other reported preconcentration methods. The results are shown in Table 11. As can be seen, the proposed procedure shows a limit of detection comparable to or better than the others. Likewise, wide linear dynamic range and high enrichment factor, which are better in most cases or are comparable with reported methods in other cases and high selectivity would make this method suitable for measuring the concentration of mercury in various samples.

Table 9. The application of proposed method for analysis of natural water and urine samples (units of all data are ng mL^{-1}, N=3).

Sample	Hg added	Hg found ET-AAS method	Hg found SPE-CV-AAS (Recovery%)	T Value
Tap water	0	BDL[a]	0.95 ± 0.01[b]	-
(Sanandaj, Kurdistan,	0.5	BDL	1.43 ± 0.03 (96)	-
IRAN)	5	5.95 ± 0.1	5.93 ± 0.05(91)	0.31[c]
River water	0	BDL	1.23 ± 0.02	-
(Sepid Rood, Astaneh	0.5	BDL	1.75 ± 0.02(104)	-
Ashrafieh, Gilan, IRAN)	5	6.18 ± 0.08	6.22 ± 0.03(91)	0.81
Sea water	0	13.68 ± 0.06	13.62 ± 0.05	1.33
(Caspian sea, Rudsar,	0.5	14.18 ± 0.07	14.12 ± 0.06(100)	1.13
Gilan, IRAN)	2	15.67 ± 0.2	15.61 ± 0.05(80)	0.5
Urine	0	3.81 ± 0.05	3.85 ± 0.04	1.08
(Child without filled teeth	0.5	4.31 ± 0.06	4.36 ± 0.06(102)	1.02
by amalgam)	5	8.77 ± 0.1	8.85 ± 0.06(100)	1.19
Urine	0	8.97 ± 0.03	9 ± 0.02	1.44
(Male with 2 filled teeth	0.5	9.45 ± 0.07	9.48 ± 0.03(96)	0.68
by amalgam)	5	13.95 ± 0.1	14.02 ± 0.03(91)	1.16

[a] Below detection limit.

[b] Mean ± standard deviation.

[c] $t = (\bar{x}_1 - \bar{x}_2) / s_p (1 / n_1 + 1 / n_2)$, Student's t-test with probability 95% = 2.78 (Degree of freedom = 6 – 2).

Table 10. The application of proposed method for analysis of fish samples (units of all data are μg g^{-1}, N=3).

Fish sample	English name	Hg added	Hg found ET-AAS Method	Hg found SPE-CV-AAS method (Recovery%)	T-Value
SiyahKoli	Caspian Vimba	0	0.4 ± 0.05[a]	0.41 ± 0.02	0.32[b]
(Shalman Rood,		0.1	0.49 ± 0.06(	0.52 ± 0.02(110)	0.96
Shalman,Gilan, IRAN)		0.5	0.88 ± 0.06	0.93 ± 0.03(87)	1.29
Kapor	Caspian Grass	0	0.53 ± 0.04	0.54 ± 0.03	0.35
(Shalman Rood, Shalman,	Carp	0.1	0.62 ± 0.05	0.65 ± 0.019(110)	1.02
Gilan, IRAN)		0.5	1.03 ± 0.04	1.05 ± 0.02(85)	0.77
Sefid	Caspian Kutum	0	0.71 ± 0.06	0.73 ± 0.03	0.52
(Caspian sea,Kiyashahr		0.1	0.82 ± 0.05	0.85 ± 0.03(90)	0.89
seaport,Gilan, IRAN)		0.5	1.23 ± 0.07	1.26 ± 0.02(88)	0.71

[a]Mean ± standard deviation.

[b]$t = (\bar{x}_1 - \bar{x}_2) / s_p (1 / n_1 + 1 / n_2)$, Student's t-test with probability 95% = 2.78 (Degree of freedom = 6 – 2).

Table 11. Characteristic performance data obtained by using the proposed and other reported methods for mercury (II) determination.

Analytical technique	LOD (ng L^{-1})	LDR (ng L^{-1})	Ef[a]	RSD (%)	Ref.
SPE-CV-AAS	12	20-1200	333	3.9	4
SPE-CV-AAS	2.5	-	25	<6	29
DLLME[c]-CV-AAS	30	500-100000	310	4	30
SPE-CV-AAS	10	100-30000	28	3.4	31
SPE-CV-AAS	1.87	6.25-310	128	2.98–4.45	32
SPE-GC-MS	1	3-800	250	6.3	33
CPE[d]-HPLC- ICP-MS	4	50-10000	42	1.08	34
SPE-CVAAS	160	-	100	2.2	35
CPE-UV-Vis	830	5000-80000	33	0.27	36
SPE[b]-CV-AAS	2.3	10- 1600	70	2	This work

[a]Enrichment factor; [b]Solid-phase extraction; [c]Dispersive liquid–liquid microextraction[d]Cloud point extraction.

4. CONCLUSIONS

In the present work, synthesis a new selective and sensitive sorbent was presented and described for the first time for determination of mercury (II) in water, fish and urine samples. Optimization of extraction was done based on the chemometrics methods such as design of experiments (DOE) that evaluate the interactions of possible influencing factors on treatment efficiency with a limited number of experiments and reaching the true optimum. Desorption and reuse experiments indicated that the adsorbent could be regenerated and reused almost without any loss of adsorption capacity for 70 times.

REFERENCES

[1] Y. Gao, Z. Shi, Z. Long, P. Wu, C. Zheng and X. Hou, Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry, *Microchem. J.* 103 (2012) 1-14.

[2] C. Chiou, S. Jiang and K.S.K. Danadurai, Determination of mercury compounds in fish by microwave-assisted extraction and liquid chromatography-vapor generation-inductively coupled plasma mass spectrometry, *Spectrochim. Acta B* 56 (2001) 1133-1142.

[3] I.M. Dittert, T.A. Maranhao, D.L.G. Borges, M.A. Vieiraa, B. Welz and A.J. Curtius, Determination of mercury in biological samples by cold vapor atomic absorption spectrometry following cloud point extraction with salt-induced phase separation, *Talanta* 72 (2007) 1786-1790.

[4] N. Pourreza, H. Parham, A.R. Kiasat, K. Ghanemi and N. Abdollahi, Solid phase extraction of mercury on sulfur loaded with N-(2-chloro benzoyl)-N′-phenylthiourea as a new adsorbent and determination by cold vapor atomic absorption spectrometry, *Talanta* 78 (2009) 1293-1297.

[5] S. Hassanpoor and G. Khayatian, Combination of Directly Suspended Droplet Microextraction and Flame Atomic Absorption Spectrometry for Determination of Trace Amounts of Iron and Copper, *J. Braz. Chem. Soc.* 25 (2014) 734-742.

[6] G. Khayatian, M. Hashemi and S. Hassan-poor, A new cloud point microextraction method for preconcentration and determination of copper in water samples, *Indian j. Chem. Technol.* 22 (2015) 338-343.

[7] G. Khayatian, S. Hassanpoor, F. Nasiri and A. Zolali, Preconcentration, determination and speciation of iron by solid-phase extraction using dimethyl (E)-2-[(Z)-1-acetyl)-2-hydroxy-1-propenyl]-2-butenedioa-te, *Quim. Nova.* 35 (2012) 535-540.

[8] E. Zolfonoun, A. Rouhollahi and A. Semnani, Solid-phase extraction and determination of ultra trace amounts of lead, mercury and cadmium in water samples using octadecyl silica membrane disks modified with 5, 5′-dithiobis (2-nitrobenzoic acid) and atomic absorption spectrometry, *Int. J. Environ. Anal. Chem.* 88 (2008) 327-336.

[9] R. Gao, Z. Hu, X. Chang, Q. He, L. Zhang, Z. Tu and J. Shi, Chemically modified activated carbon with 1-acylthiosemicarbazide for selective solid-phase extraction and preconcentration of trace Cu(II), Hg(II) and Pb(II) from water samples, *J. Hazard. Mater.* 172 (2009) 324-329.

[10] N. Pourreza and K. Ghanemi, Determination of mercury in water and fish samples by cold vapor atomic absorption spectrometry after solid phase extraction on agar modified with 2-mercaptobenzimidazole, *J. Hazard. Mater.* 161 (2009) 982-987.

[11] M. Faraji, Y. Yamini and M. Rezaee, Extraction of trace amounts of mercury with sodium dodecyle sulphate-coated magnetite nanoparticles and its determination by flow injection inductively coupled plasma-optical emission spectrometry, *Talanta* 81 (2010) 831-836.

[12] V. Romero, I. Costas-Mora, I. Lavilla and C. Bendicho, Cold vapor-solid phase microextraction using amalgamation in different Pd-based substrates combined with direct thermal desorption in a modified absorption cell for the determination of Hg by atomic absorption spectrometry, *Spectrochim. Acta B* 66 (2011) 156-162.

[13] X. Chai, X. Chang, Z. Hu, Q. He, Z. Tu and Z. Li, Solid phase extraction of trace Hg(II) on silica gel modified with 2-(2-oxoethyl)hydrazine carbothioamide and determination by ICP-AES, *Talanta* 82 (2010) 1791-1796.

[14] Q. Wu, X. Chang, Q.He, Y. Zhai, Y. Cui, X. Huang, Silica gel modified withdiaminothiourea as selective solid-phase extractant for determination of Hg(II) in biological and natural water samples, *Int. J. Environ. Anal. Chem.* 88 (2008) 245-254.

[15] A.S. Pereira, G. Ferreira, L. Caetano, M.A.U. Martines, P.M. Padilha, A. Santos and G.R. Castro, Preconcentration and determination of Cu(II) in a fresh water sample using modified silica gel as a solid-phase extraction adsorbent, *J. Hazard. Mater.* 175 (2010) 399-403.

[16] R. Leardi, Experimental design in chemistry: A tutorial, *Anal. Chim. Acta* 652 (2009) 161-172.

[17] A. N. Panagiotou, V. A. Sakkas and T. A. Albanis, Application of Chemometric Assisted Dispersive Liquid–Liquid Microextraction to the Determination of Personal Care Products in Natural Waters, *Anal. Chim. Acta* 649 (2009) 135-140.

[18] F. Nasiri, A. Zolali and S. Asadbegi, Solvent-free one-pot synthesis of 2, 2' dithioxo-[5, 5] bithiazolidine-4 ,4'-dions, *J. Heterocyclic Chem.* 53 (2016) 989-992.

[19] J. Fan, Y. Qin, C. Ye, P. Peng and C. Wu, Preparation of the diphenylcarbazone-functionalized silica gel and its application to on-line selective solid-phase extraction and determination of mercury by flow-injection spectrophotometry, *J. Hazard. Mater.* 150 (2008) 343-350.

[20] N.M. Bahadur, T. Furusawa, M. Sato, F. Kurayama, I.A. Siddiquey and N. Suzuki, Fast and facile synthesis of silica coated silver nanoparticles by microwave irradiation, *J. Colloid Interf. Sci.* 355 (2011) 312-320.

[21] C.N.R. Rao and R. Venkataraghavan, The C=S stretching frequency and the "-N-C=S bands" in the infrared, *Spect. Chim. Acta* 18 (1962) 541-547.

[22] B. Kešeljević, J. Huremović and J. Sulejmanović, *Glas. hem. tehnol. Bosne Herceg,* 2012, 38, 31.

[23] T. Ahmadzadeh Kokya and K. Farhadi, Optimization of dispersive liquid–liquid microextraction for the selective determination of trace amounts of palladium by flame atomic absorption spectroscopy, *J. Hazard. Mater.* 169 (2009) 726-733.

[24] E.D. Morgan, *Chemometrics: Experimental design, Analytical chemistry by open learning,* John Wiley & Sons, England (1991).

[25] D. Brynn Hibbert, Exprimental design in choromatography: Atutorial review, *J. Chromatogr.* B, 910 (2012) 2-13.

[26] R. Khani, F. Shemirani and B. Majidi, Combination of dispersive liquid–liquid microextraction and flame atomic absorption spectrometry for preconcentration and determination of copper in water samples, *Desalination* 266 (2011) 238-243.

[27] P. Araujo, F. Couillard, E. Leirnes, K. Ask, A. Bøkevoll and L. Frøyland, Experimental design considerations in quantification experiments by using the internal standard technique: Cholesterol determination by gas chromatography as a case study, *J. Chromatogr. A* 1121 (2006) 99-105.

[28] R.G. Pearson, Hard and soft acids and bases, *J. Am. Chem. Soc.* 85 (1963) 3533-3539.

[29] M. Tuzena, I. Karaman, D. Citak and M. Soylak, Mercury(II) and methyl mercury determinations in water and fish samples by using solid phase extraction and cold vapour atomic absorption spectrometry combination, *Food Chem. Toxicol.* 47 (2009) 1648-1652.

[30] E. Stanisz, J. Werner and H. Matusiewicz, Mercury species determination by task specific ionic liquid-based ultrasound-assisted dispersive liquid–liquid microextraction combined with cold vapour generation atomic absorption spectrometry, *Microchem. J.* 110 (2013) 28-35.

[31] E. Vereda Alonso, M.T. Siles Cordero, A. García de Torres, P. Ca˜ nada Rudner and J. M. Cano Pavón, Mercury speciation in sea food by flow injection cold vapor atomic absorption spectrometry using selective solid phase extraction, *Talanta* 77 (2008) 53-59.

[32] M. Soleimani, M.S. Mahmodi, A. Morsali, A. Khani and M.G. Afshar, Using a new ligand for solid phase extraction of mercury, *J. Hazard. Mater.* 189 (2011) 371-376.

[33] J. Munoz, M. Gallego and M. Valcarcel, Solid-phase extraction–gas chromatography–mass spectrometry using a fullerene sorbent for the determination of inorganic mercury(II), methylmercury(I) and ethylmercury(I) in surface waters at sub-ng/ml levels, *J. Chromatogr A* 1055 (2004) 185-190.

[34] H. Chena, J. Chen, X. Jin and D. Wei, Determination of trace mercury species by high performance liquid chromatography–inductively coupled plasma mass spectrometry after cloud point extraction, *J. Hazard. Mater.* 172 (2009) 1282-1287.

[35] Y. Zhai, S.E. Duan, Q. He, X. Yang and Q. Han, Solid phase extraction and preconcentration of trace mercury(II) from aqueous solution using magnetic nanoparticles doped with 1,5-diphenylcarbazide, *Microchim. Acta* 169 (2010) 353-360.

[36] A. Niazi, T. Momeni-Isfahani and Z. Ahmari, Spectrophotometric determination of mercury in water samples after cloud point extraction using nonionic surfactant Triton X-114, *J. Hazard. Mater.* 165 (2009) 1200-1203.