

ORIGINAL RESEARCH PAPER

Application of Nanoanalysis: Amine-Functionalized Mesoporous Silica nanoparticles for Mercury Speciation in Human Samples

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ABSTRACT

Mercury (Hg) causes hazardous cumulative effects in humans; like central nervous system disorders, hypertension and chromosomal aberrations. Therefore, due to high toxicity and bioaccumulation factor, mercury determination and speciation in human blood is very important. A sensitive, accurate, precise and inexpensive method was demonstrated for preconcentration and speciation of ultra-trace mercury in human blood samples. The amine-functionalized mesoporous silica nanoparticles (NH₂-MSNPs) as solid phase sorbent was prepared and investigated in a new and simple dispersive-ionic liquid-micro-solid phase extraction (D-IL- μ -SPE) procedure for rapid speciation and determination of trace inorganic and organic mercury [Hg²⁺, (CH₃Hg)⁺ and (C₂H₅Hg)⁺] in human blood samples. Under the optimal conditions, the linear range, limit of detection and preconcentration factor were obtained 0.05-9.8 μ g L⁻¹, 15 ng L⁻¹ and 9.6 for human blood samples, respectively (Peak Area, RSD<5%). The reusability and batch adsorption capacity of the MSNPs and NH₂-MSNPs were also obtained 25 and 159 mg g⁻¹, respectively. The developed method was successfully applied for validation of methodology by standard reference materials (SRM).

Keywords: Amine-functionalized mesoporous silica nanoparticles, Dispersive-ionic liquid-micro-solid phase extraction, Mercury species, Human blood

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INTRODUCTION

Mercury (Hg) is one of the most toxic elements for human and environment and has different forms, elemental mercury (Hg⁰), inorganic mercury (Hg²⁺) and organic mercury (CH₃-Hg and C₂H₅-Hg). The organic mercury is more toxic than other forms in human cells. Mercury has toxic as; effects on the human body, such central nervous system disorder (CNSD), neurological

diseases, kidney disorders, respiratory problems, dermatitis, cancer, hypertension, and chromosomal aberrations. In addition, subjective symptoms, including nervousness, fatigue, depression, tremor, insomnia and memory impairments have been already reported. The metabolic rates of mercury species in clinical specimens are very important factor. The speciation and determination of mercury species in different clinical specimens such as; urine (Hg²⁺, Hg⁰) and whole blood (Hg²⁺, Hg⁰, Me-Hg, Et-Hg) is necessary because of their toxicity. With respect to,

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the Inorganic and organic mercury speciation in the blood samples was achieved. So, mercury speciation in the blood is more important than urine samples. In addition, methylmercury is almost always higher than the ethyl mercury if the human body is exposed to both types [1-4]. The permissible level of inorganic mercury in drinking water and whole blood is less than $6 \mu\text{g L}^{-1}$ and $10 \mu\text{g L}^{-1}$, respectively. The threshold limit values of mercury exposure in air reported by occupational safety and health administration (OSHA), national institute of occupational safety and health (NIOSH) and American conference of governmental industrial hygienists (ACGIH) and were 0.1 mg m^{-3} , 0.05 mg m^{-3} and 0.025 mg m^{-3} , respectively [5-8]. In view of the above facts, it is essential to have reliable analytical methods based on speciation analysis, which can differentiate between chemical forms in blood samples to diagnose risks of toxicity. Many analytical methods for speciation and determination of mercury in biological samples were used [9, 10]. With other analytical methods, mercury speciation was achieved by decomposing of R-Hg to Hg^{2+} in biological samples. UV light was used for speciation and determination of mercury. In the absence of UV light R-Hg does not decompose to Hg^{2+} , even in the presence of acids. R-Hg is not easily decomposed by heating in the presence of acid concentrations; however, with UV light the decomposition of R-Hg to Hg^{2+} takes place immediately, depending on the intensity of UV light.

In addition, sensitive analytical techniques were used for determination of mercury in blood samples such as; high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) [11], inductively coupled plasma atomic emission spectrometry (ICP-AES) [12], Cold vapor/hydride generation atomic absorption spectrometry (CV-AAS) [13], hydride generation and atomic fluorescence spectrometry (CV-AFS) [14, 15], and electro-thermal atomic absorption spectrometry (ET-AAS) [16], and gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) [17]. Among them, CV-AAS is a conversional spectrometry in chemistry laboratory and widely applied to determination of mercury ions in biological samples. The CV-AAS cannot be used individually for speciation of Hg in human blood samples. But on account of extremely low concentration and high matrices effects of mercury in biological samples, a separation and preconcentration step is required [18-20].

Recently, a microextraction technique based on dispersive-micro-solid phase extraction (D- μ -SPE), has been reported for extracting of target analytes from biological human samples [20]. The extraction

recovery in D- μ -SPE method depended on dispersion of the solid sorbent particles into the sample solution and phase separation by centrifugation [21]. Also, Sample clean-up and the extraction step are carried out simultaneously and so, it is suitable for the extraction in complex matrices [22-24]. The nature and properties of the solid sorbent are of prime importance in D- μ -SPE. Nanomaterials possess a large surface area and short diffusion route, which may result in high extraction efficiency and rapid extraction dynamics [20, 24, 25].

The aim of the present study is to develop a new analytical method for rapid speciation, preconcentration and determination of trace Hg^{+2} and R-Hg (Me-Hg and Et-Hg) in human blood samples based on the combination of D-IL- μ -SPE technique and CV-AAS detection method. The mesoporous silica nanoparticles (MSNPs) was prepared, then chemically functionalized with amine groups (NH_2 -MSNPs), and finally used as adsorbent in the presented method with no chelating agent. Hexyl-3-methylimidazolium hexafluorophosphate was used as NH_2 -MSNPs trapping agent in the sample solution. All main factors affecting the extraction process were investigated and optimized.

EXPERIMENTAL

Material and Method

Determination of mercury (Hg) was performed with a GBC atomic absorption spectrometer (GBC 932- HG3000-AUS, Australia) equipped with a flow injection cold vapor module (CV-AAS), deuterium-lamp, background corrector, Hg hollow-cathode lamp, and a circulating reaction loop circulating cooling unit. The pH values of the solutions were measured by a digital pH meter (Metrohm, model 744, Herisau, Switzerland). A Hettich centrifuge (model EBA 20, Hittech, Germany) and an ultrasonic bath with heating system (Tecno-GAZ SPA, Italy) were used throughout this study. A Multi-Wave 3000 microwave-assisted UV system (MUV, Anton Paar, Graz, Austria) was used for converting R-Hg to Hg^{+2} in standard solution and blood samples. All reagents used were of the highest purity available and at least of analytical reagent grade available and purchased from Merck (Darmstadt, Germany), unless otherwise stated. All aqueous solutions were prepared in ultra-pure deionized water ($R \geq 18 \text{ M}\Omega \text{ cm}^{-1}$) from Milli-Q plus water purification system (Millipore, Bedford, MA, USA). Hg^{+2} standard stock solution (1000 mg L^{-1} in 1% nitric acid, 250 mL) was purchased from Fluka, Buchs, Switzerland. The $(\text{CH}_3\text{Hg})^+$ and $(\text{C}_2\text{H}_5\text{Hg})^+$ 1000 mg L^{-1} stock solutions were prepared by dissolving appropriate amounts of their chloride in the smallest possible volume of methanol and diluting

to volume with deionized water. A 0.6% (w/v) sodium borohydride reagent solution was prepared daily by dissolving an appropriate amount of NaBH_4 in 0.5% (w/v) sodium hydroxide and used as a reducing agent. Tetraethyl ortho-silicate (TEOS, $(\text{C}_2\text{H}_5\text{O})_4\text{Si}$, CN: 8006580025), triethanolamine (TEAH₃, $\text{C}_6\text{H}_{15}\text{NO}_3$, CASN: 102-71-6), cetyltrimethylammonium bromide (CTAB, $\text{C}_{19}\text{H}_{42}\text{BrN}$, CASN: 57-09-0), Nitric acid (HNO_3), hydrochloric acid (HCl), sodium acetate (NaOAc), sodium hydroxide (NaOH), potassium hydroxide (KOH) and all of the other reagents used for experiments and analysis were of analytical grade and purchased from Merck, Darmstadt, Germany. The working standard solutions were prepared daily by diluting the stock solutions of mercury ions [Hg^{2+} , $(\text{CH}_3\text{Hg})^+$ and $(\text{C}_2\text{H}_5\text{Hg})^+$] with deionized water prior to analysis with the proposed method. The pH adjustments were made using appropriate buffer solutions including sodium phosphate ($\text{H}_3\text{PO}_4/\text{NaH}_2\text{PO}_4$, 0.1 mol L⁻¹) for pH 2-3, ammonium acetate ($\text{CH}_3\text{COOH}/\text{CHCOONH}_4$, 0.1 mol L⁻¹) for pH 4-6, sodium borate (NaBO_2/HCl , 0.1 mol L⁻¹) for pH 7, and ammonium chloride ($\text{NH}_3/\text{NH}_4\text{Cl}$, 0.1 mol L⁻¹) for pH 8-10. The general procedure for synthesis of amine-functionalized mesoporous silica nanoparticles (NH_2 -MSNPs) is the atrane route, in which the presence of the polyalcohol is the key to balancing the hydrolysis and condensation reaction rates. In a typical synthesis, TEOS (tetraethyl ortho-silicate) was added to predetermine amounts of TEAH₃ (triethanolamine). The solution was heated up to 140°C under vigorous stirring. After cooling down to 90°C, CTAB (cetyltrimethylammonium bromide) was added to this solution. For the functionalization of calcined MSNPs with amine groups, 1.2 g of amine compound and 2 g of calcined MSNPs were added to appropriate amount of toluene and refluxed for 24 hour at 80°C.

Humans sampling

For sampling, all glass tubes were washed with a 0.5 M of HNO_3 solution for at least 24 hour and thoroughly rinsed many times with ultrapure water (DW) before using. As mercury species concentrations in the

human body are very low, even minor contamination at any stage of sampling, sample storage and handling, or analysis has the potential to affect the accuracy of the results. Heparin free mercury is commonly used as anticoagulants in human blood samples. The blood collection tube with heparin was aliquoted into Eppendorf (10 mL) tubes and kept at -20°C for one week. For analysis, 20 µL of pure heparin was added to blood sample (10 mL) of petrochemical worker, Iran.

Characterization of MSNPs and NH_2 -MSNPs

The FT-IR spectra patterns of MSNPs and NH_2 -MSNPs in the range of 4000-500 cm⁻¹ are shown in Figure 1. These results confirmed the presence of amine (NH_2) group on MSNPs surface. The XRD patterns of calcined MSNPs and NH_2 -MSNPs are shown in Figure 2. There are three resolved diffraction peaks in XRD patterns of NH_2 -MSNPs and MSNPs, which can be indexed as the (100), (110), (200) and (210) reflections associated with hexagonal symmetry (d_{110} and d_{200} were overlapped with each other). However, these peaks are broad, which are the characteristic of mesoporous materials synthesized via atrane route. After the attachment of organic groups on the silica wall of MSNPs, the main three diffraction peaks are still clear which means that functionalization procedure did not have what effect on the structural order of MSNPs.

The specific surface area (S_{BET}) of MSNPs and NH_2 -MSNPs were calculated from the linear part of the BET equation that was 863 m² g⁻¹ and 626 m² g⁻¹, respectively. Decreasing of BET surface area, pore volume, and pore diameter of NH_2 -MSNPs in comparison with initial MSNPs are due to the grafting of aminosilane on silica walls. The unit cell parameter (a_0) and the average pore wall thickness (W_t) of the sorbents were calculated by the equations of $a_0 = 2 \cdot d_{100} / \sqrt{3}$ and $W_t = a_0 - (d_p / 1.05)$, respectively, where d_p is the pore diameter of adsorbent and d_{100} is obtained from XRD diffractograms. As shown in Table 1, these two parameters are almost constant for both the sorbents.

Table 1. Textural properties of MSNPs and NH_2 -MSNPs

Sample	SBET ^a (m ² /g)	d_{sp} ^b (nm)	d_{lp} ^c (nm)	V_{sp} ^d (cm ³ /g)	V_{lp} ^e (cm ³ /g)	a^f (nm)	W_t^g (nm)
MSNPs	863	2.67	52.2	0.42	0.84	5.59	2.92
NH_2 -MSNPs	626	2.62	42.2	0.27	0.41	5.59	2.97

^aBET specific surface area, ^bdiameter of small pores, ^cdiameter of large pores, ^dVolume of small pores, ^eVolume of large pores, ^fUnit cell parameter obtained from XRD diffractograms ($2d_{100}/\sqrt{3}$), ^gWall thickness(nm) obtained by following equation: $W_t = a - (d_p/1.05)$.

The SEM was performed to illustrate the morphology and particle size distribution of the calcined NH_2 -MSNPs. As shown in Figure 3, NH_2 -MSNPs has a highly porous morphology and the mesoporous silica particles are in nanometer range (25 nm). Moreover, functionalization did not lead to bulky silica nanoparticles. TEM image also illustrates the pore structure of NH_2 -MSNPs. As shown in Figure 4, the mesopores are clearly visible in the silica nanoparticles and particle size of the samples is in the nanometer range around 20 to 50 nm as those observed in SEM image.

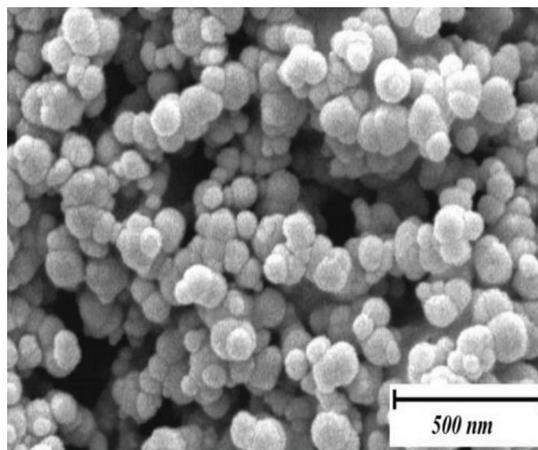


Fig. 3. The SEM of NH_2 -MSNPs.

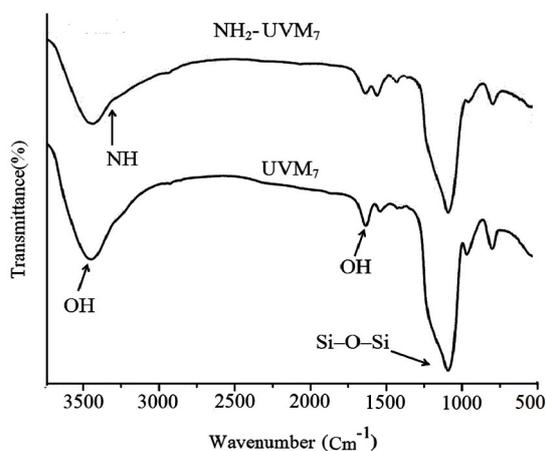


Fig. 1. The FT-IR of MSNPs/ NH_2 -MSNPs.

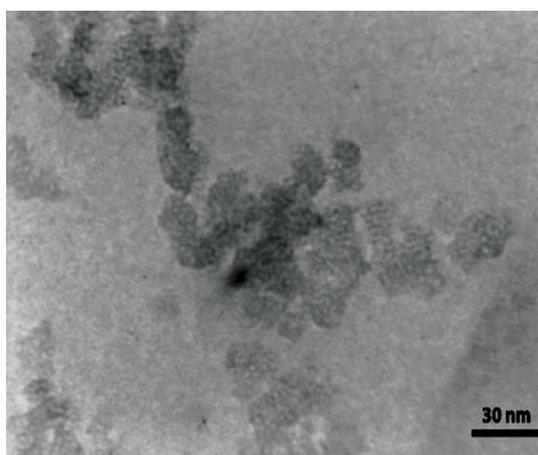


Fig. 4. The TEM of NH_2 -MSNPs.

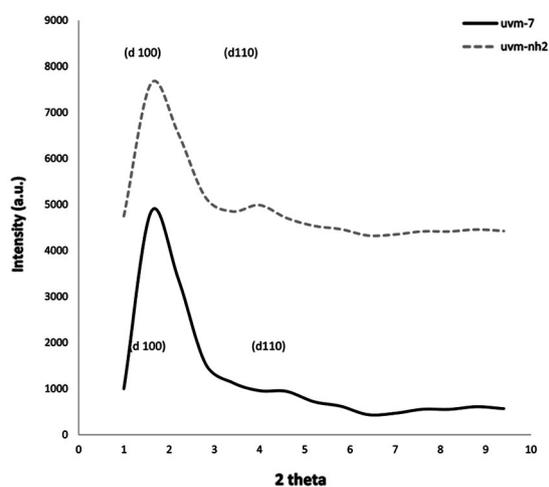


Fig. 2. The XRD of MSNPs / NH_2 -MSNPs.

General procedure (D-IL- μ -SPE)

The D-IL- μ -SPE procedure was performed with 10 mL of blood sample or standard aqueous solution containing Hg^{+2} and/or R-Hg at pH of 8.5 with sodium phosphate buffer solution ($\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, 0.4 M). The procedure of the proposed method was performed as follows: 10 mL of the standard aqueous solution containing Hg^{+2} and/or R-Hg with concentration in the range of 0.1-9.5 $\mu\text{g L}^{-1}$ or 10 mL of blood sample transferred into the 10 mL of the centrifuge tube. Meanwhile, 0.2 g of [HMIM][PF₆] dispersed in 200 μL acetone was mixed with 5 mg NH_2 -MSNPs sorbent and rapidly injected by a syringe into the sample solution. The resulting mixture was shaken in an ultrasonic bath for 5 min at 25°C (50 kHz, 100 W). Hg^{+2} and/or R-Hg species were extracted and preconcentrated by NH_2 -MSNPs ($\text{M}^+ \cdot \text{NH}_2$). The sorbent was trapped with [HMIM][PF₆] and

the solution was centrifuged for 2 min. The NH_2 -MSNPs/IL suspension was settled down in bottom of the conical centrifuge tube and the aqueous phase was removed with a transfer pipette. Finally, mercury species retained on the sorbent were eluted by adding 0.5 mL of HNO_3 (0.2 M) and the eluent phase was separated from NH_2 -MSNPs/IL phase by centrifuging for 2.0 min. Finally, the eluent phase was diluted with deionized water up to 1 mL the concentration of Hg^{+2} were analysed by CV-AAS. Ultraviolet (UV) light and microwave were used as the appropriate sources for rapid decomposing of R-Hg to Hg^{+2} . Total mercury was determined when the remaining eluent was put on microwave vessel (150°C, 4 mL Conc. HNO_3 , UV, 15 min) and all forms of R-Hg converted to Hg^{+2} . Finally, the concentration of R-Hg is simply calculated by subtracting of Hg^{+2} concentration and total mercury concentration (T-Hg).

RESULT AND DISCUSSION

The matrix effect (%ME) in human urine samples were calculated as extracted analyte (Hg) from a human blood matrix to extract analyte from a matrix-free solution (Standard mercury solution) by D-IL- μ -SPE technique and CV-AAS detection method which was shown in equation 1 (EQ1). The recovery was obtained by equation 2.

% ME = (Peak area of the Hg extraction in human matrix) / (Peak area of the Hg extraction in matrix free solution) $\times 100$ (EQ1)

$$\text{Recovery \%} = \frac{(C_i - C_f)}{C_i} \times 100 \quad (\text{EQ2})$$

Elemental analysis

Elemental analysis provides further evidence for the amount of amine functional groups grafted on NH_2 -MSNPs. The yield of functionalization can be calculated using the elemental analysis results (the ratio of nitrogen content of the amine-functionalized NH_2 -MSNPs divided by the amount of nitrogen of the triethoxysilpropylamine used for functionalization). The nitrogen content was 4.83 wt %.

Effect of pH

In the D-IL- μ -SPE technique, the pH of the sample solution is an important parameter to obtain quantitative recoveries of mercury ions, because it affects the surface charge of the adsorbent or function group of ligand and

the degree of ionization for speciation of the adsorbent or analyte. So, the influence of sample pH on the recovery efficiency of Hg ions by NH_2 -MSNPs and MSNPs was investigated in pH ranges between 2 to 10 by using buffered sample solutions containing 1-5 $\mu\text{g L}^{-1}$ of Hg^{+2} and R-Hg. The recovery percentages of Hg^{+2} increased from pH 8 to 9, and Hg ions were quantitatively recovered (>98%) at pH=8.5 (Figure 5).

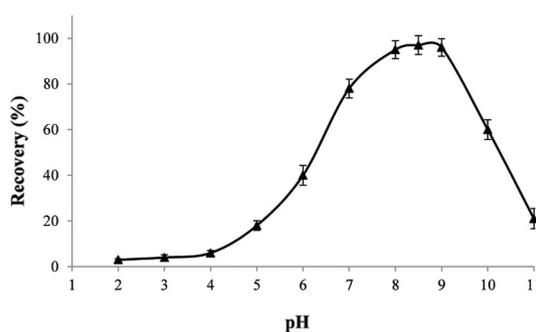


Fig. 5. The effect of pH.

Effect of the amount of adsorbent and ILs

In this study, the effect of NH_2 -MSNPs and $[\text{HMIM}][\text{PF}_6]$ on the recoveries of Hg ions was investigated. The various amounts of NH_2 -MSNPs and $[\text{HMIM}][\text{PF}_6]$ in the ranges of 1 to 15 mg and 0.1-0.5 g was studied, respectively. The extraction efficiency of arsenic speciation with a mixture of NH_2 -MSNPs and $[\text{HMIM}][\text{PF}_6]$ were obtained more than 4 mg and 0.15 g respectively (R>98%). So, 5 mg of NH_2 -MSNPs and 0.2 g of IL were considered as the optimum amounts of adsorbent and IL (Figure 6).

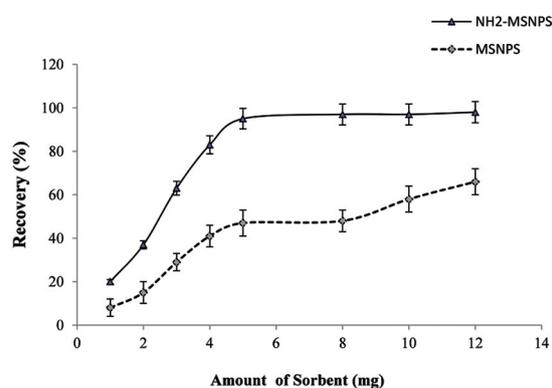


Fig. 6. The effect of amount of sorbent.

Method validation

The procedure provides novel and interesting approach using the NH_2 -MSNPs for extraction of organic and inorganic mercury species from human biological samples. In order to obtain optimum speciation conditions and quantitative recoveries of inorganic and organic mercury species with good sensitivity and precision, the presented D-IL- μ -SPE system was optimized for various analytical parameters. Moreover, in order to optimization of effecting parameters, standard solutions containing different concentrations of Hg^{+2} and R-Hg in the range of 0.1–9.0 $\mu\text{g L}^{-1}$ were

examined. The developed D-IL- μ -SPE method was applied to the determination of trace organic and inorganic mercury species in blood and standard samples. The obtained results, as the average of three separate determinations, for Hg^{2+} , R-Hg, and total Hg are shown in Tables 2 and 3 for blood samples. The accuracy of the results was verified by analyzing the spiked samples with known concentration of Hg^{+2} and R-Hg can be seen from Tables 2 and 3 a good agreement was obtained between the added and measured Hg^{+2} amount, which confirms the accuracy of the procedure and its independence from the matrix effects.

Table 2. Analytical results of spike of Hg^{+2} and R-Hg concentration in human blood samples

Sample	Added ($\mu\text{g L}^{-1}$)		Found ^a ($\mu\text{g L}^{-1}$)		Recovery (%)	
	Hg (II)	R-Hg	Hg^{+2}	R-Hg	Hg^{2+}	R-Hg
Blood A	-----	-----	1.25 ± 0.04	0.65 ± 0.02	-----	-----
	1.0	-----	2.27 ± 0.11	0.62 ± 0.03	102	-----
	-----	1.0	1.22 ± 0.06	1.63 ± 0.05	-----	98
Blood B	-----	-----	0.82 ± 0.03	0.43 ± 0.02	-----	-----
	0.2	-----	1.01 ± 0.05	0.45 ± 0.03	95	-----
	-----	0.2	0.84 ± 0.04	0.64 ± 0.03	-----	105
Blood C	-----	-----	5.65 ± 0.22	2.24 ± 0.09	-----	-----
	2.0	-----	7.58 ± 0.34	2.19 ± 0.11	97	-----
	-----	2.0	5.61 ± 0.26	4.26 ± 0.18	-----	101

^a Mean of three determinations \pm confidence interval (P=0.95, n=5).

Table 3. Analytical results of spike of Hg^{+2} and R-Hg concentration by standard solution samples

Standard Sample	Added ($\mu\text{g L}^{-1}$)		Found ^a ($\mu\text{g L}^{-1}$)		Recovery (%)	
	Hg (II)	R-Hg	Hg^{+2}	R-Hg	Hg (II)	R-Hg
Std. 1	-----	-----	1.02 ± 0.06	1.08 ± 0.05	-----	-----
	0.5	-----	1.54 ± 0.07	0.98 ± 0.04	104	-----
	-----	0.5	1.05 ± 0.05	1.56 ± 0.08	-----	96
Std. 2	-----	-----	3.05 ± 0.14	2.98 ± 0.12	-----	-----
	3.0	-----	5.93 ± 0.36	3.04 ± 0.16	96	-----
	-----	3.0	2.96 ± 0.17	6.08 ± 0.31	-----	103
Std. 3	-----	-----	3.02 ± 0.16	2.04 ± 0.06	-----	-----
	5.0	-----	7.92 ± 0.38	2.05 ± 0.07	98	-----
	-----	5.0	2.86 ± 0.17	6.88 ± 0.29	-----	97

^a Mean of three determinations \pm confidence interval (P=0.95, n=5).

Table 4. Validation of developed method with biological standard reference material (SRM)

Sample	Certified ($\mu\text{g L}^{-1}$)		Found a ($\mu\text{g L}^{-1}$)		Recovery (%)	
	Hg (II)	R-Hg ^b	Hg (II)	R-Hg	Hg (II)	R-Hg
^a NIST, SRM	9.00 \pm 1.30	9.60 \pm 1.50	8.65 \pm 0.37	9.41 \pm 0.29	96	98

^a Mean of three determinations \pm confidence interval ($P=0.95$, $n=5$), ^b R-Hg include of CH₃Hg and C₂H₅Hg, ^c SRM 995 c, level 3.

In order to validate the method described, the certified standard reference materials, NIST-SRM 995c (mercury species in caprine blood) was analyzed and the results were given in Table 4. Analytical results of the SRM samples were satisfactorily in agreement with the certified values. Moreover, a good agreement was obtained between added and found values of the mercury species in spiked SRM samples. The recoveries of spiked samples for both organic and inorganic Hg species were ranged from 96% to 102%, which demonstrated that the developed method was satisfactory for mercury analysis.

This procedure was performed by adding various amounts of the interfering ions to 10 mL of standard sample solution containing 1.0-9.0 $\mu\text{g L}^{-1}$ of Hg²⁺, (CH₃Hg)⁺ and (C₂H₅Hg)⁺. Taking as criterion for interference the deviation of the recovery more than $\pm 5\%$, the results showed that most of the probable concomitant cations and anions have no considerable effect on the recovery efficiencies of mercury species ions in optimized conditions.

CONCLUSION

A simple, fast and reliable method were developed for speciation and determination of trace Hg²⁺ and R-Hg in human blood samples. The method was obtained based on D-IL- μ -SPE technique and CV-AAS detection method. By using of [HMIM][PF₆] ionic liquid as trapping agent of the mercury-loaded NH₂-MSNPs sorbent is a rapid single step, reducing the sample preparation and separation time (without filtration) and sorbent loss. The US-D-IL- μ -SPE method compares with alternative methods for extraction of mercury from different samples. To the best of our knowledge the solid phase extraction and determination of inorganic and organic Hg in biological fluids has not been explored so far, and no work has been documented in blood samples by NH₂-MSNPs. The proposed method shows easy and new extraction procedure of trace mercury levels (Hg²⁺ and R-Hg),

using cheaper and user-friendly instruments. The developed US-D-IL- μ -SPE method shows good linear ranges, low LOD, and RSD (%) values as well as satisfactory preconcentration factor values (PF) for the determination of Hg²⁺ and R-Hg ions. The LODs obtained were perfectly adequate for the analyzed biological samples. These results demonstrate high sensitivity and precision of the proposed method.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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