In-vitro speciation of molybdenum (II, VI) in human biological samples based on thiol-functionalized mesoporous silica nanoparticles and hexyl-methylimidazolium tris-pentafluoroethyl-trifluorophosphate

Roya Ashouri ^{a,b} and Seyed Alireza Hajiseyed Mirzahosseini ^{b,*}

^aDepartment of Environmental science, Science and Research Branch, Islamic Azad University, Tehran, Iran

^bDepartment of Environmental Engineering, Faculty of Natural Resources and Environment, Science and Research Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO:

Received 30 May 2020

Revised form 25 Jul 2020

Accepted 20 Aug 2020

Available online 30 Sep 2020

Keywords:

Molybdenum, Speciation,
Human serum,
Ionic liquid,
Thiol-functionalized bimodal
mesoporous silica nanoparticles;
Dispersive-ionic liquid-micro-solid
phase extraction

ABSTRACT

Molybdenum (Mo) ions enter to human body from the diet or drinking waters and have a potentially toxic effect on human. The thiol-functionalized mesoporous silica nanoparticles (HS-MSNPs) was used for determination and speciation of Mo (II, VI) in human biological samples by dispersive ionic liquid-micro-solid phase extraction (DIL- μ -SPE) coupled to electrothermal atomic absorption spectrometry (ET-AAS). Firstly, the mixture of HS-MSNPs (15 mg), the hydrophobic ionic liquid (1-Hexyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate; [HMIM][T(PFE)PF₃]) and acetone injected to 10 mL of human blood and serum samples. After shaking for 5 min, the Mo(II) and Mo(VI) ions were extracted with thiol group of MSNPs at pH 6 and 2, respectively and collected through IL in bottom of conical tube by centrifuging. Then, the Mo(II,VI) ions were back-extracted from HS-MSNPs with eluent based on changing pH, and remained solutions were determined by ET-AAS after dilution with DW up to 0.5 mL, separately. So, the total of Mo(T-Mo) was simply calculated by the summation of Mo(II) and Mo(VI) content. In optimized conditions, the linear range (LR), the limit of detection and enrichment factor (EF) for Mo(II) and Mo(VI) were obtained (0.41-3.82 $\mu\text{g L}^{-1}$; 0.48-4.55 $\mu\text{g L}^{-1}$), (0.1 $\mu\text{g L}^{-1}$; 0.12 $\mu\text{g L}^{-1}$) and (19.6; 16.5) for 10 mL of human blood samples, respectively (Mean of RSD%=3.3). At optimized pH, the adsorption capacities of the HS-MSNPs for Mo(II) and Mo(VI) was obtained 68.7 mg g^{-1} and 55.8 mg g^{-1} , respectively. In purposed study, a new analytical method for rapid speciation and determination of trace amount of Mo (II, VI) was used in human blood and serum samples. The developed method was successfully validated by ICP-MS analysis.

1. Introduction

Molybdenum as inorganic material belongs to

transition metal. Molybdenum (Mo) exists in environment with different oxidation numbers and complex compounds. The high concentration of Mo has toxic effects in humans and cause to many diseases in human body like central nervous system,

Corresponding Author: Alireza Hajiseyed Mirzahosseini

Email: mirzahosseini@gmail.com

<https://doi.org/10.24200/amecj.v3.i03.115>

liver and renal disorder. Molybdenum has low concentration in seawater and high concentrations in kidney, liver, and adrenals of human [1]. In human body, the concentration of Mo in serum is almost $0.6 \mu\text{g L}^{-1}$ and depends on dietary intake. Normal serum concentrations are between $0.3\text{--}2.0 \mu\text{g L}^{-1}$. Normal whole blood concentrations are between $0.6\text{--}4.0 \mu\text{g L}^{-1}$ in unexposed peoples and $1.2\text{--}4.8 \mu\text{g L}^{-1}$ in exposed peoples [2]. The molybdate can be chemically adsorbed onto positively charged iron, aluminium or manganese oxides [3]. Therefore, high molybdenum intake causes a secondary copper deficiency and is called molybdenosis or hypocuprosis [4]. Molybdenum toxicity caused to diarrhea, anorexia, graying of hair, anemia and these symptoms are readily reversed by copper supplementation. The tetrathiomolybdates interact with copper and caused to the treatment of copper disorders such as Wilson's disease [5]. The intracellular pool of cysteine is relatively small as compared to the glutathione (GSH) [6]. By oxidizing, cysteine is oxidized to cystine and so, the cysteine in plasma has low concentration between $10\text{--}25 \mu\text{M}$ as compared to cystine concentration from 50 to $150 \mu\text{M}$ [7]. The cells have different transport for cysteine (Cys) and cystine by different membrane carriers and both of them can be reaction with Mo and complex with Mo as R-Mo [8]. Molybdenum cofactor deficiency (Moco) is a metabolic disorder and cause to defects in the biosynthesis of Moco leading to loss of activity of Mo enzymes [9]. Molybdenum has seen in brain and can be measured in urine, blood, and cerebrospinal fluid (CSF). Mo(II) locates in the mitochondria and cytoplasm of cells. Mo helps to involve the metabolism pathway of sulfate and sulfite oxide deficiency. Mo(VI) as MoO_4^{2-} or MoO_3 binds strongly to amino acids, enzymes (aldehyde Oxidase, sulfite oxidase and xanthine oxidase), metallothionein (MT), molybdenum cofactor (Mo(VI), molybdopterin), cysteine (Cys), proteins (Pr) and metabolized in the brain and other tissues. So, the organic Mo(R-Mo) is important in human body and must be determined [10-12]. Many developed methods included,

flame atomic absorption spectrometry (F-AAS) [13], the stripping voltammetry [14], the UV visible spectrophotometer [15-17] were used for determination Mo in different matrixes such as water and human biological samples. As difficulty matrix in human blood samples, we have to use the extraction techniques for preparation of human blood samples. The ionic liquids (ILs) as an organic salts have the various advantages such as thermal stability, high viscosity and good solvent for separation phase. The ILs as a green solvent were used for extraction or separation ions from liquid phases. Recently, the sample preparation such as liquid-liquid microextraction (LLME) [15, 17], flow injection chemiluminescence method combined with controlled potential electrolysis technique [18] and solid phase extraction based on inductively coupled plasma-optical emission spectrometry (ICP-OES (SPE) [19-21] reported for extraction Mo from samples.

In this study, the speciation of Mo(II) and Mo(VI) in human blood samples was obtained based on HS-MSNPs nanostructure by DIL- μ -SPE procedure. The concentration of Mo was determined by ET-AAS and hydrophobic Ionic liquid (1-Hexyl-3-methylimidazolium tris (pentafluoroethyl) trifluorophosphate; [HMIM][T(PFE)PF₃]) can be used for separating and collecting of HS-MSNPs adsorbent from liquid phase. In optimized conditions, a simple, fast and sensitive procedure was demonstrated for speciation and determination of trace Mo(II) and Mo(VI) in human biological samples.

2. Experimental

2.1. Instruments

Determination of Mo was performed with a GBC atomic absorption spectrometer (GBC 932-HG3000- Australia) equipped with a graphite furnace module (GF-AAS) and deuterium-lamp background corrector. A hollow cathode lamp of Mo with wavelength of 313.3 nm (7 mA , and 0.2 nm slit) was used. The temperature programming adjusted as the ash and atomize points of 1200°C and 2700°C , respectively. Argon is the preferred as

a carrier gas for Mo with flowrate of 0.4 ng mL^{-1} . The concentration of standard solutions of Mo ($20 \text{ }\mu\text{L}$, $30 \text{ }\mu\text{g L}^{-1}$) has a 0.3 absorption (Abs) by ET-AAS. The pH values of the solutions were measured by a digital pH meter (PeakTech, model P5310, China, CAS No: 9027802000). A refrigerated centrifuge, from 1.5 per 2 mL microtubes was used for separation IL from solution. Long and short blood collection tubes for 15 mL and 50 mL of tubes were used with RCF of $2.6 \times g$ (4000 rpm , model S300TR, Japan). The ultrasonic bath with heating system has been designed for performance, control, durability and reliability to disperse nanoparticles in liquid phase. (Branson, Tomas Scientific, Swedesboro, U.S.A.). The crystal structure studies of the solids were carried out by X-ray diffractions (PW 1840, Phillips X-ray diffractometer, Netherland) with Cu-K_α radiation source.

2.2. Reagents

All reagents with high purity and analytical grade were purchased from Merck (Darmstadt, Germany). The ethanol, acetone and toluene all were purchased from Merck, Germany. All aqueous solutions were prepared in ultra-pure deionized water from water purification system (Millipore, Bedford, MA, USA). The powder standard of MoO_3 (VI) (CAS: 1313-27-5) was purchased from Sigma, Aldrich, Germany (1 g of powder dissolved in one liter of DW as 1000 mg L^{-1} in 1% nitric acid). The Molybdenum (II) acetate dimer as di-molybdenum tetraacetate (CAS: 14221-06-8) was purchased from Merck, Germany. The general procedure for synthesis of thiol-functionalized bimodal mesoporous silica nanoparticles (HS-MSNPs) was done in RIPI, Iran. The reagents; HCl , CH_3COOH , tetraethylortho-silicate (TEOS, CAS: 8006580025), cetyltrimethylammonium bromide (CTAB, CAS: 57-09-0), and 3-mercaptopropyltriethoxysilane was purchased from Sigma Aldrich (Darmstadt, Germany). 1-Hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF₆]; CAS No.: 304680-35-1), 1-Hexyl-methylimidazolium tris(pentafluoroethyl) trifluorophosphate ([HMIM]

[T(PFE)PF₃]; CAS No: 713512-19-7) and 1-Butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆]; CAS No: 174501-64-5) purchased from Sigma, Germany.

2.3. Synthesis of thiol functionalized MSNPs

The general procedure for synthesis of MSNPs is the atrane route, in which the presence of the polyalcohol is the key to balancing the hydrolysis and condensation reaction rates [22]. After preparation MSNPs, 1.4 g of 3-Mercaptopropyltriethoxysilane ($\text{C}_9\text{H}_{22}\text{SO}_3\text{Si}$) and 1.5 g of pure MSNPs were added to appropriate volume of toluene and then the mixture was refluxed for two days and followed by filtering and washing of product with ethanol and water for many times. The thiol functionalized MSNPs (HS-MSNPs) was created after drying at 80°C for 8-10 h.

2.4. Characterization

Functional groups of SH on MSNPs were analyzed by Fourier transform infrared spectrophotometer (FTIR, IFS 88, Bruker Optik GmbH, Germany) using KBr pelleting method in the $4000\text{--}300 \text{ cm}^{-1}$. The scanning/ transmission electron microscopy was obtained in this study. Scanning electron microscopy (SEM, Phillips, PW3710, Netherland) was used for morphology and surface image analysis of the sorbents. The nanoparticles size for HS-MSNPs was examined by transmission electron microscopy (TEM, CM30, Philips, Netherland). The X-ray diffraction (XRD) patterns continued with the Shimadzu XRD which are designed with the concept of provide solution to XRD analysis by ease of use and versatility. Basic system with high precision goniometer can be varied with optional items to adapt to the purpose (MAXima-X XRD-7000).

2.5. DIL- μ -SPE Procedure

The DIL- μ -SPE Procedure was performed with 10 mL of human blood and serum samples. For adjusting of the parameters, the standard aqueous solution containing Mo(II) and Mo(VI) with concentration in the range of $0.5\text{--}3.0 \text{ }\mu\text{g L}^{-1}$

was used in optimum pH of 6 and 2 with buffer solution, respectively before moved to centrifuge conical tube. After adjusting pH, the HS-MSNPs (15 mg), 0.15 g of [HMIM][T(PFE)PF₃] and 200 μ L of acetone were mixed and rapidly injected by a syringe into the sample solution. Then, the sample was dispersed in solution by ultrasonic bath for 7 min at room temperature (50 kHz, 100 W). Mo(II) and Mo(VI) species were extracted and preconcentrated by HS-MSNPs at pH of 6 and 2, separately. The loaded sorbent (Mo—HS-MSNPs) was collected with IL by centrifuging at 4000 rpm for 5 min. The adsorbed Mo(II) and Mo(VI) on HS-MSNPs/IL was back-extracted in different pH (acidic pH=2.5 for Mo²⁺ and pH=7.5 for Mo⁶⁺) and concentration of Mo(II) and Mo(VI)

ions determined by ET-AAS (Fig. 1). Iso, the total Mo was calculated by the summation of Mo(II) and Mo(VI) content [Mo(II)+ Mo(VI)]. By DIL- μ -SPE Procedure, the matrix effect in human blood and serum samples were achieved by ratio of extracted Mo(II) or Mo(VI) in human blood sample to standard solution as a matrix-free solution which was shown in below equation. Matrix Effect (%) = (Signal of the Mo extraction in blood / signal of Standard solution) $\times$ 100

3. Results and discussion

3.1. The XRD and FT-IR analysis

The XRD patterns of calcined HS-MSNPs and MSNPs are shown in Figure 2. There are three resolved diffraction peaks in XRD patterns,

Fig. 1. The speciation of Mo(II) and Mo(VI) based on HS-MSNPs by DIL- μ -SPE Procedure

Fig.2. The XRD patterns of of MSNPs and HS- MSNPs

which can be indexed as the (100), (110), (200) and (210) reflections associated with hexagonal symmetry (d110 and d200 were overlapped with each other). After the attachment of HS on the silica wall of MSNPs, the main three diffraction peaks are still clear and similar which means that functionalization of HS on MSNPs did not had worth effect on the structural order of MSNPs (Fig. 2). The FTIR spectra patterns of MSNPs and HS- MSNPs was shown in Figure 3. A peak of absorption about 3550 cm^{-1} and 1640 cm^{-1} related to OH bonding, the peak at 1100 cm^{-1} showed silicon dioxide (SiO_2) and peak at 2565

cm^{-1} was confirmed the SH group on the walls of MSNPs.

3.2. SEM and TEM imaging

The SEM was performed to illustrate the morphology and particle size distribution of the calcined HS-MSNPs. As shown in Fig. 4a, HS-MSNPs has a highly porous morphology and the mesoporous silica particles are in nanometer range (40 nm). Moreover, functionalization of HS did not lead to bulky silica nanoparticles. TEM image also illustrates the size and pore structure of HS-MSNPs. As shown in Fig. 4b, the mesoporous

Fig. 3. The FT-IR spectra patterns of MSN and HS- MSN

Fig. 4. The SEM (a) and TEM (b) of HS-MSNPs

Fig. 5. The effect of pH on speciation of Mo(II) and Mo(VI) ions by HS-MSNPs adsorbent

are clearly visible in the silica nanoparticles and particle size of the samples is in nanometer range around 25 to 50 nm.

3.3. The pH and sample volume optimizations

By DIL- μ -SPE technique for determination and speciation of Mo in human blood samples, the pH must be studied and optimized. The pH effect on quantitative recoveries of Mo(II) and Mo(VI) ions by changing of the surface charge of the thiol group on HS-MSNPs adsorbent as the negative or positive charges which can attract with Mo(II) and Mo(VI) ions. So, the influence of sample pH on the extraction efficiency of Mo(II) and Mo(VI) ions was investigated in pH ranges from 1 to 11 by using favorite buffered solutions and containing 0.4-3.0 $\mu\text{g L}^{-1}$ of Mo(II) and Mo(VI). The results showed us, the Mo(II) and Mo(VI) ions were efficiently extracted at pH of 5-7 and pH of 1-3. Therefore, the pH 2 and 6 was used as optimum pH for extraction of Mo(II) and Mo(VI) ions from blood samples by HS-MSNPs adsorbent (Fig. 5). Also, the effect of blood sample volume for extraction of Mo(II) and Mo(VI) ions based on HS-MSNPs adsorbent was studied from 2-25 mL. The results of extraction showed us, the best recovery was obtained for 12 mL of blood samples. So, 10 mL of blood samples was used for further study.

3.4. Speciation mechanism

The thiol functionalized mesoporous silica nanoparticles (HS-MSNPs) can be adsorbed Mo(II) and Mo(VI) ions from blood samples. The thiol groups can be deprotonated (SH^-) more than pH=5 and Mo(II) can be absorbed as a rule of opposite charge at optimized pH. So, the interaction of HS groups of sorbent with cationic form of Mo^{2+} was occurred at pH 5-7. In addition, the positive charge of thiol (SH_2^+) was obtained at acidic pH (1-3) and so, Mo(VI) anions adsorbed on HS-MSNPs by positive charged of thiol group in human blood samples. Therefore, the pH of 2 and 6 was selected as optimum pH for extraction and speciation Mo(II) and Mo(VI) ions from blood samples by DIL- μ -SPE procedure.

3.5. Effect of the amount of adsorbent and ILs

In this work, the effect of HS-MSNPs mass on the recoveries of Mo(II) and Mo(VI) ions was investigated. So, the various amounts of HS-MSNPs in the ranges of 2 to 20 mg of adsorbent were studied and optimized. The results showed us, the extraction efficiency for speciation of Mo(II) and Mo(VI) ions were obtained more than 14 mg of HS-MSNPs. By same conditions, the recovery of extraction for MSNPs was obtained less than 28% and 23% for Mo(II) and Mo(VI) ions, respectively. So, 15 mg of HS-MSNPs were used for further works (Fig. 6).

Fig. 6. The effect HS-MSNPs adsorbent on speciation of Mo(II) and Mo(VI) ions by DIL- μ -SPE Procedure

Fig. 7. The effect of ILs on speciation of Mo(II) and Mo(VI) ions by DIL- μ -SPE Procedure

Moreover, the effect of kind and amount of ILs were studied by proposed procedure. So, the different amount of hydrophobic ionic liquids such as; 1-Hexyl-3-methylimidazolium hexafluorophosphate; [HMIM][PF₆], Hexyl-methylimidazolium tris(pentafluoroethyl) trifluorophosphate; [HMIM][T(PFE)PF₃] and Butyl 3-methyl imidazolium hexafluorophosphate; [BMIM][PF₆] were studied between 0.05-0.25 g. The results showed, 0.15 g of [HMIM][T(PFE)PF₃] had the favorite recovery more than 95% as compared to others. The recovery for extraction Mo(II) and Mo(VI) ions for 1-Hexyl-3-methylimidazolium hexafluorophosphate and 1-Butyl-3-methylimidazolium hexafluorophosphate

were obtained 79% and 74%, respectively. Therefore, 0.15 g of [HMIM][T(PFE)PF₃] was selected as optimum amount of IL for this work (**Fig. 7**).

3.6. Effect of matrix

The effect of interference ions concentration for extraction of Mo(II) and Mo(VI) ions must be studied by DIL- μ -SPE Procedure. So, the effect of some interfering ions for the determination of Mo(II) and Mo(VI) ions were investigated. The procedure was done for 10 ml of sample containing 3.5 $\mu\text{g L}^{-1}$ of Mo(II) and Mo(VI) ions with different concentration of interference ions between 0.5-2 mg L^{-1} . Based on results, the interference ions in blood samples such

as Cu^{2+} , Mn^{2+} , Mg^{2+} , Zn^{2+} , CO_3^{2-} , PO_4^{3-} and HCO_3^- do not interfere for extraction of Mo(II)/ Mo(VI) ions under optimized conditions (**Table 1**).

3.7. Validation in real samples

The DIL- μ -SPE method was used to determine Mo(II) and Mo(VI) ions in 10 mL of human blood samples.

For validation in real samples, the serum and blood samples were spiked to demonstrate the accuracy and precision of the method for determination of Mo(II) and Mo(VI) ions. The spiked samples is satisfactorily recoveries between 95-102% and was confirmed using addition method (**Table 2**).

By procedure, the calibration curve for Mo(II) and

Table 1. The effect of interferences ions on extraction of Mo(II) and Mo(VI) ions in blood samples by DIL- μ -SPE Procedure

Blood Interfering ions (BII)	Mean ratio ($C_{\text{BII}}/C_{\text{Mo}}$)		Recovery (%)	
	Mo(II)	Mo(VI)	Mo(II)	Mo(VI)
Mn^{2+} , Cd^{2+} ,	800	650	97.7	98.2
Cr^{3+} , Al^{3+} ,	700	700	98.8	96.5
V^{3+}	850	500	97.1	97.6
Ca^{2+} , Mg^{2+}	900	1000	98.9	99.2
Na^+ , K^+ ,	1100	1100	98.2	98.5
Zn^{2+} , Cu^{2+}	750	600	97.5	96.4
I^- , Br^- , F^- , Cl^-	1200	1100	99.4	98.3
CO_3^{2-} , SO_3^{2-}	900	1000	97.5	98.6
HCO_3^- , PO_4^{3-}	800	700	97.0	96.4
Ni^{2+} , Co^{2+}	450	600	95.7	97.2
Pb^{2+}	350	500	98.1	98.4
Hg^{2+}	50	80	96.3	97.2

Table 2. Validation of methodology based on HS-MSNPs adsorbent for speciation of Mo(II) and Mo(VI) ions in serum and blood samples by spiking to real samples

Sample	Added ($\mu\text{g L}^{-1}$)		Found ($\mu\text{g L}^{-1}$)			Recovery (%)	
	Mo (II)	Mo(VI)	Mo (II)	Mo(VI)	T-Mo	Mo (II)	Mo(VI)
Blood A	-----	-----	0.72 ± 0.04	0.56 ± 0.03	1.28 ± 0.06	-----	-----
	0.5	-----	1.20 ± 0.05	0.54 ± 0.02	1.74 ± 0.08	96.0	-----
	-----	0.5	0.69 ± 0.04	1.07 ± 0.04	1.76 ± 0.09	-----	102
Blood B	-----	-----	1.74 ± 0.03	0.83 ± 0.03	2.57 ± 0.12	-----	-----
	1.5	-----	3.19 ± 0.16	0.80 ± 0.04	3.99 ± 0.19	96.6	-----
	-----	1.0	1.76 ± 0.04	1.81 ± 0.05	3.57 ± 0.17	-----	98
Serum C	-----	-----	0.92 ± 0.05	2.05 ± 0.11	2.97 ± 0.16	-----	-----
	1.0	-----	1.89 ± 0.09	1.99 ± 0.09	3.88 ± 0.21	97.0	-----
	-----	2.0	0.89 ± 0.04	3.98 ± 0.22	4.87 ± 0.25	-----	96.5
Serum D	-----	-----	2.12 ± 0.11	1.33 ± 0.06	3.45 ± 0.18	-----	-----
	1.5	-----	3.60 ± 0.17	1.29 ± 0.05	4.89 ± 0.24	98.6	-----
	-----	1.5	2.16 ± 0.12	2.76 ± 0.15	4.92 ± 0.25	-----	95.3

^a Mean of three determinations $\pm$ SD (P= 0.95, n=5)

Table 3. Validation of results for determination of Mo(II) and Mo(VI) ions in standard solutions by ICP-MS analysis and compared to DIL- μ -SPE method

Sample	CRM ($\mu\text{g L}^{-1}$)		Found ^a ($\mu\text{g L}^{-1}$)		Recovery (%)	
	Mo (II)	Mo(VI)	Mo (II)	Mo(VI)	Mo (II)	Mo(VI)
Standard	1.56 $\pm$ 0.01	2.07 $\pm$ 0.04	1.51 $\pm$ 0.08	1.97 $\pm$ 0.12	96.8	95.2
Standard	3.25 $\pm$ 0.02	1.84 $\pm$ 0.03	3.27 $\pm$ 0.14	1.79 $\pm$ 0.09	100.6	97.3

^a Mean of three determinations $\pm$ SD (P= 0.95, n=5)

Table 4. Validation of results for determination of total Mo(T-Mo) ions in blood and serum samples by ICP-MS analysis and compared to DIL- μ -SPE method

Sample	ICP-MS ($\mu\text{g L}^{-1}$)		^a DIL- μ -SPE ($\mu\text{g L}^{-1}$)		Recovery (%)	
	T-Mo		T-Mo		T-Mo	
	Blood	Serum	Blood	Serum	Blood	Serum
Sample A	2.43 $\pm$ 0.03	2.72 $\pm$ 0.05	2.33 $\pm$ 0.11	2.75 $\pm$ 0.14	95.8	101.1
Sample B	1.17 $\pm$ 0.02	1.29 $\pm$ 0.03	1.15 $\pm$ 0.05	1.24 $\pm$ 0.07	98.3	96.1
Sample C	3.36 $\pm$ 0.07	3.71 $\pm$ 0.08	3.25 $\pm$ 0.14	3.62 $\pm$ 0.16	96.7	97.6

^a Mean of three determinations $\pm$ SD (P= 0.95, n=5)

Mo(VI) ions was linear between 0.41-3.82 $\mu\text{g L}^{-1}$ and 0.48–4.55 $\mu\text{g L}^{-1}$, respectively. In addition, the ICP-MS analysis was used for speciation of Mo(II) and Mo(VI) ions in standard solutions (Table 3). Also, the ICP-MS analysis was used for determination of total Mo(T-Mo) in human blood and serum samples as certified reference material (CRM) which compared to DIL- μ -SPE method. The ICP-MS analysis confirmed the accuracy and precision of proposed procedure for determination and speciation of Mo(II) and Mo(VI) ions in human blood samples (Table 4).

4. Conclusions

In this research, a simple, sensitive, accurate and precise method was used to demonstrate the separation /speciation and determination of trace Mo(II) and Mo(VI) ions in human blood samples. By DIL- μ -SPE procedure, the effect of main factors on extraction process such as pH and amount of adsorbent and IL were optimized. The mean of enrichment factor and recovery was obtained 18.05 and 98.6 %, respectively. The mean of LOD and LR was achieved 0.11 $\mu\text{g L}^{-1}$ and between 0.445-4.19 μg

L^{-1} , respectively in optimized pH. Based on Table 4, the results of DIL- μ -SPE method for determination of total Mo in blood and serum samples were comparable to ICP-MS technique. The adsorption capacities of the HS-MSNPs and MSNPs for total Mo was obtained 62.3 mg g^{-1} and 18.2 mg g^{-1} , respectively. Also, the relative standard deviation (RSD%) for speciation and determination of trace Mo(II) and Mo(VI) ions in human blood samples was obtained 2.3 and 2.8, respectively. The validation of the methodology was confirmed by spiking to real samples and ICP-MS analysis in standard solution or human blood samples.

5. Acknowledgment

The authors wish to thank from Department of Environmental science, Science and Research Branch, Islamic Azad University, Tehran, Iran

6. References

- [1] J. L. Burguera, M. Burguera, Molybdenum in human whole blood of adult residents of the Merida State (Venezuela), J. Trace Elem. Med. Biol., 21 (2007) 178–183.

- [2] J. Oh, S. H. Shin, R. Choi, S. Kim, H. D. Park, S. Y. Kim, S. A. Han, W. J. Koh, and S. Y. Lee, Assessment of 7 trace elements in serum of patients with nontuberculous mycobacterial lung disease, *J. Trace Elem. Med. Biol.*, 53 (2019) 84-90.
- [3] J. R. Turnlund, W. R. Keyes, Plasma molybdenum reflects dietary molybdenum intake, *J. Nutr. Biochem.*, 15 (2004) 90-95.
- [4] G. N. George, I. J. Pickering, H. H. Harris, J. Gailer, D. Klein, J. Lichtmannegger, K. H. Summer, Tetrathiomolybdate Causes Formation of Hepatic Copper-Molybdenum Clusters in an Animal Model of Wilson's Disease, *J. Am. Chem. Soc.*, 125 (2003) 1704-1705.
- [5] G. J. Brewer, The use of copper-lowering therapy with tetrathiomolybdate in medicine *Expert Opin. Investig. Drugs*, 18 (2009) 89-97.
- [6] C. Picó, F. Serra, Biomarkers of Nutrition and Health: New Tools for New Approaches, *J. Nutr.*, 11 (2019) 1092.
- [7] G. Wu, Y. Z. Fang, S. Yang, J. R. Lupton, N. D. Turner, Glutathione metabolism and its implications for health, *J. Nutr.*, 134 (2004) 489-492.
- [8] Y. Xiong, Y. Xiong, Y. Wang, Z. Wang, A Zhang, Inhibition of Glutathione Synthesis via Decreased Glucose Metabolism in Stored RBCs, *Cell Physiol. Biochem.*, 51(2018) 2172-2184.
- [9] J.L. Johnson M. Duran, Molybdenum cofactor deficiency and isolated sulfite oxidase deficiency, 8th ed. McGraw-Hill, New York, pp. 3163-3177, 2001.
- [10] G. Schwarz, A. Ali Belaidi, Molybdenum in human health and disease, *Metal Ions Life Sci.*, 13 (2013) 415-450.
- [11] K. Pytlakowska, K. Kocot, M. Pilch, M. Zubko, Ultrasound-assisted dispersive micro-solid phase extraction using molybdenum disulfide supported on reduced graphene oxide for energy dispersive X-ray fluorescence spectrometric determination of chromium species in water, *Microchim. Acta*, 187 (2020) 542.
- [12] G. R. Helz, T. P. Vorlicek, Precipitation of molybdenum from euxinic waters and the role of organic matter, *Chem. Geo.*, 509 (2019) 178-193.
- [13] M. Sanchez, D. Gazquez, P. Garcia, Determination of molybdenum by atomic-absorption spectrometry after separation by 5,5'-methylenebis(salicylohydroxamic acid) extraction and further reaction with thiocyanate and tin (II), *Talanta*, 38 (1991) 747-52.
- [14] V. Arancibi, C. R. R. Margarit, E. Aliag, E. Stegmann, Fast and highly sensitive method for molybdenum(VI) determination by catalytic adsorptive stripping voltammetry *Sensors Actuators B: Chem.*, 258 (2018) 612-620.
- [15] M. B. Febrian, T. S. Mulyati, A. Suherman, Spectrophotometric determination of Mo content in ^{99m}Tc solution Via Mo-TGA-KSCN complexes formation, *Indonesian J. Nucl. Sci. Technol.*, 19 (2018) 71-80.
- [16] B. Spasova, C. Kuesters, B. Stengel, Spectrophotometric determination of molybdenum-containing compounds in aqueous glucose solutions, *Chem. Eng. Technol.*, 41(2018) 1776-1782.
- [17] S. Bhardwaj, L.R. Kakkar, A highly sensitive and selective spectrophotometric determination of molybdenum using o-Phenanthroline in presence of thiocyanate, *Der Chem Sin.*, 4 (2013) 32-43.
- [18] J. X. Du, J. J. Li, L. J. Yang, J. R. Lu, Sensitive and selective determination of molybdenum by flow injection chemiluminescence method combined with controlled potential electrolysis technique, *Anal. Chim. Acta*, 481 (2003) 239-244.
- [19] S. Hassanpour, M. Taghizadeh, Rapid and Selective Separation of Molybdenum Ions using a novel magnetic Mo(VI) ion imprinted polymer: a study of the adsorption properties, *RSC Adv.*, 6 (2016) 100248-100261.
- [20] F. Ardestani, M. H. Hosseini, M. Taghizadeh,

- M. R. Pourjavid, M. Rezaee, Synthesis and characterization of nanopore MoVI-imprinted polymer and its application as solid phase for extraction, separation and preconcentration of molybdenum ions from water samples, *J. Braz. Chem. Soc.*, 27 (2016) 1279–1289.
- [21] A. Bagheri, M. Behbahani, M. M. Amini, O. Sadeghi, A. Tootoonchi, Z. Dahaghin, Preconcentration and separation of ultra-trace palladium ion using pyridinefunctionalized magnetic nanoparticles, *Microchim. Acta*, 178 (2012) 261–268.
- [22] H. Shirkhanloo, A. Khaligh, H.Z. Mousavi, A. Rashidi, Ultrasound assisted-dispersive-micro-solid phase extraction based on bulky amino bimodal mesoporous silica nanoparticles for speciation of trace manganese (II)/(VII) ions in water samples. *Microchem. J.*, 124 (2016) 637-645.