Biol Trace Elem Res (2018) 183:173–181
DOI 10.1007/s12011-017-1118-8

Speciation of As(III)/As(V) and Total Inorganic Arsenic in Biological Fluids Using New Mode of Liquid-Phase Microextraction and Electrothermal Atomic Absorption Spectrometry

Lida Haghnazari[1] · Nezam Mirzaei[2,3] · Hossein Arfaeinia[4] · Kamaladdin Karimyan[5] · Hooshmand Sharafi[6] · Nazir Fattahi[6]

Received: 14 June 2017 / Accepted: 2 August 2017 / Published online: 12 August 2017

Abstract In this paper, a new extraction method based on countercurrent liquid–liquid microextraction (CLLME) has been developed for the extraction and preconcentration of inorganic arsenic (iAs) in plasma and urine samples prior to their analysis by electrothermal atomic absorption spectrometry (ETAAS). In this method, firstly, 5 ml of water is added to the extraction vessel. Then 30.0 μl of the extracting solvent is added to it in order for the extracting solvent to be placed in the narrow-necked vessel. In total, 10 ml of a standard solution or a pretreated real sample is added to the sample container and it is connected to the extraction vessel via a connector. While opening the embedded valve at the bottom of the sample container and the one in the extraction vessel, the sample solution flows into the extracting solvent with the same flow rate, leading to the successful extraction of metal ligand into the extracting organic solvent. Under the optimum conditions, calibration curves are linear in the range of 0.1–50 μg l^{-1}, and limit of detections (LODs) are in the range of 0.03–0.05 μg l^{-1}. The enhancement factor and enrichment factor were in the range of 220–240 and 198–212, respectively. Repeatability (intra-day) and reproducibility (inter-day) of method based on seven replicate measurements of 5.0 μg l^{-1} of arsenic were in the range of 2.3–3.5% and 4.0–5.7%, respectively. The applicability of the proposed CLLME and ETAAS methods was demonstrated by analyzing the iAs in spiked urine and plasma samples. The obtained recoveries of the arsenic in the range of 92–107% indicated the excellent capability of the developed method for speciation of arsenic from plasma and urine samples.

Keywords Arsenic speciation · Countercurrent liquid–liquid microextraction · Extraction solvent lighter than water · Biological analysis

✉ Nazir Fattahi
nazirfatahi@yahoo.com

1 Department of Clinical Biochemistry, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

2 Department of Environmental Health Engineering, Faculty of Health, Kashan University of Medical Sciences, Kashan, Iran

3 Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

4 Department of Environmental Health Engineering, School of Public Health, Bushehr University of Medical Sciences, Bushehr, Iran

5 Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

6 Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran

Introduction

Arsenic (As) is widely distributed in environment as various species which can enter and accumulate in living organisms to cause the toxic effect [1]. Although the biotic source of As is geological, human activities such as mining, burning of fossil fuels, and pesticide application also cause As pollution. Toxicity of As depends not only on the total concentration but also on the chemical forms in which this analyte is present [2]. Inorganic arsenic (iAs) species including arsenite (As(III)) and arsenate (As(V)) are more toxic than their organic species such as monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA), while arsenobetaine (AsB) and arsenocholine (AsC) are considered as non-toxic arsenic compounds [3]. The toxicity of As(III) is 10–20 times higher than that of As(V), and its oxide has been shown to cause several types

of cancer [4]. Knowledge of the arsenic speciation in biological samples is important because the physiological and toxicological effects of arsenic depend on its chemical form [5]. Exposure to arsenic can cause a variety of adverse health effects, including dermal changes, respiratory, cardiovascular, gastrointestinal, genotoxic, mutagenic, and carcinogenic effects [6]. Therefore, it is necessary to determine the inorganic As species in order to estimate its toxic effects and health risks.

Nowadays, many kinds of conventional analytical techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) [1, 3, 7, 8], inductively coupled plasma atomic emission spectrometry (ICP-AES) [9], inductively coupled plasma optical emission spectrometry (ICP-OES) [10], hydride generation atomic absorption spectrometry (HG-AAS) [11–14], and electotermal atomic absorption spectrometry (ETAAS) [2, 4, 5, 13–19] have been used for the determination of the low concentration levels of arsenic. ETAAS is still being used because it combines a fast analysis time, a relative simplicity, a cheaper cost, low sample volume requirements, and low detection limits. However, low concentration and matrix interference are two problems in detecting arsenic compounds. In this context, the development of methods for separation and preconcentration of arsenic is necessary.

Current research in analytical chemistry has focused on small-scale, simplified, efficient, and particularly environmentally friendly extraction technique. In recent years, various sample preparation methods, based on solvent microextraction approaches such as single-drop microextraction (SDME) [20], hollow fiber-based liquid-phase microextraction (HF-LPME) [1, 21, 22], dispersive liquid–liquid microextraction (DLLME) [23–26], and dispersive liquid–liquid microextraction based on solidification of a floating organic drop (DLLME-SFO) [1, 15, 16, 27] have been developed for the extraction and preconcentration of As from biological, food, and environmental samples. However, disadvantages of SDME are air bubble formation, time consuming, lake of equilibrium, and fast stirring may break up the organic solvent drop. Also, HF-LPME is time consuming, and in most cases, equilibrium is not attained even after a long time. DLLME, which is a rapid, simple, and low-cost method with a high enrichment factor, has gained immediate and considerable attention by the researchers [28–32]. The main disadvantage of the DLLME technique is the use of high-density extraction solvents, being mostly halogenated, that are generally hazardous to laboratory personnel and the environment. In DLLME-SFO, the extraction solvent used has low density and melting point near room temperature, which forms a layer on top of the aqueous sample, and can be easily collected by solidifying it at low temperature. In addition, the use of the extraction solvent with lower toxicity in DLLME-SFO is more environmentally friendly. However, it is based upon a ternary component solvent system, in which utilization of the co-solvents (disperser solvents) leads to some disadvantages such as decrease in the partition coefficients for extraction of the analytes into the extracting solvent and increase in the cost and environmental pollution.

The aim of this work was to develop a new version of LPME, namely countercurrent liquid–liquid microextraction (CLLME) and its combination with ETAAS for the extraction and preconcentration of iAs from biological samples. The extraction of target analytes was carried out using a new extraction vessel containing a narrow-necked glass tube with a valve in its bottom. Such a tube makes it possible for the solvents with lower densities than water to be applied for the extraction of organic components. Basically, water is poured into the lower part of the glass tube and the lower-density organic solvent is added to the narrow neck of the tube. The sample solution is kept in another container which has a valve at its bottom. Using a connector, the output of the sample container is connected to the input section of the narrow-necked extraction vessel. Opening both valves with the same flow simultaneously, the sample solution flows into the organic solvent and the analytes are extracted into the organic solvent. In this method, there is a diversity of choices in selecting a safe and non-toxic extracting organic solvents such as hydrocarbons and alcohols; it is not limited to the chloride organic solvents which are higher in density than water. In addition, no dispersing solvent is needed in the proposed method and no pump is also needed to carry the sample. No centrifuge is required for collecting the extraction solvent. To the best of our knowledge, for the first time, the CLLME-ETAAS is developed and applied to the extraction and preconcentration of iAs in plasma and urine samples.

Experimental

Reagents and Solutions

Stock standard solutions of As(III) and As(V) with a concentration of 1000 mg l^{-1} were obtained by dissolving appropriate amounts of As_2O_3 and Na_2HAsO_4 (Merck, Darmstadt, Germany). Working standard solutions were prepared daily by diluting the stock solution with ultrapure water. The chelating agent diethyldithiophosphoric acid (DDTP), with a density of 1.17 kg l^{-1}, all organic solvents, and other chemicals and reagents were obtained from Merck (Merk, Darmstadt, Germany) and used as received. The ultra-pure water (six times distilled) was purchased from Shahid Ghazi Company (SGC, Tabriz, Iran). A mixture of 1000 mg l^{-1} $Pd(NO_3)_2$ and 300 mg l^{-1} $Mg(NO_3)_2$ solutions (both Merck, Darmstadt, Germany) were used as chemical modifiers. Sodium thiosulfate and potassium iodide (both Merck) were added for the reduction of As(V) to the trivalent state in sample solutions in order to determine total As. A standard reference material (SRM) NIST-2669 Arsenic

Table 1 Instrumental parameters and temperature program for inorganic arsenic determination

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (L min^{-1})
Inject modifier	80	5	25	1.5
Inject sample				
Drying I	110	3	20	1.5
Drying II	240	2	10	1.5
Pyrolysis	850	20	10	1.5
AZ*	850	0	6	0
Atomization	2100	0	3	0
Cleaning	2500	0	2	2

Species in Frozen Human Urine from National Institute of Standards and Technology (NIST) was employed for the validation of the proposed method.

Instrumentation

A Model nov AA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) equipped with deuterium background correction, a transversely heated graphite tube atomizer, and an MPE 60 autosampler was used for all measurements. Deuterium-arc background correction was employed to correct non-specific absorbance. The sample injection volume was 20 μl in all experiments, and measurements were performed using integrated absorbance (peak area). Pyrolytic graphite platforms inserted into pyrolytically coated tubes were obtained from the same manufacturer (Part No.407-A81.026). The instrumental parameters and temperature program for the graphite atomizer are listed in Table 1. An arsenic hollow cathode lamp (Analytik Jena, Jena, Germany), operated at a current of 5.0 mA and a wavelength of 193.7 nm with a spectral band width of 0.8 nm, was used. Argon 99.999% was purchased from Roham Gas Co. (RGC, Arak, Iran) and was used as protected and purge gas. The pH values were measured with a Metrohm pH-meter (Model: 692, Herisau, Switzerland) supplied with a glass-combined electrode.

Sampling and Sample Preparation

Urine samples were collected from four persons (two males and two females), and plasma samples were collected from two persons (one male and one female), kindly provided by the Clinic of Imam Reza Hospital (Kernanshah, Iran). Urine and plasma samples were kept frozen at − 20 °C before analysis.

Urine samples were thawed at room temperature and homogenized by vortex. Then, 4.0 ml was centrifuged for 8 min at 4000 rpm, and the supernatant was transferred into a clean glass tube. This solution was diluted to 10.0 ml using HNO_3 (0.02 mol l^{-1}) solution for adjusting pH, ionic strength, and decreasing matrix effects.

In the preparation of plasma sample, 2.0 ml of plasma was placed in a 15-ml glass tube and 1000 μl of 15% (*w*/*v*) $ZnSO_4$ and 700 μl acetonitrile was added. The glass tube was vortexed for 10 min, maintained at 4 °C for 5 min followed by centrifugation at 4000 rpm for 8 min. Then, the supernatant was collected in the another test tube, and this solution was diluted to 10.0 ml using 0.02 mol l^{-1} HNO_3 (for adjusting pH and decreasing matrix effects). The resulting solutions were then subjected to the CLLME process.

Extraction Procedure

Firstly, 4.7 ml of water is added to the extraction vessel. Then 30.0 μl of extracting solvent is added to it in order for the extracting solvent to be placed in the narrow-necked vessel.

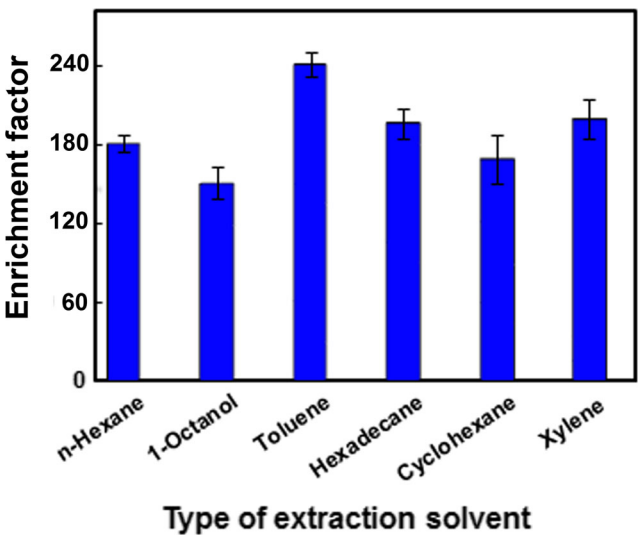

Fig. 1 Analytical absorbance of different extraction solvents evaluated for extraction of the As(III) by CLLME. Extraction conditions: sample, 10 ml diluted urine or plasma spiked with 5.0 μg l^{-1} of As(III); extraction solvent volume, 30.0 μl; flow rate, 1.0 ml min^{-1}; concentration of DDTP, 0.1% (*v*/*v*); sample solution pH, 2; room temperature and without salt addition

An aliquot of 10.0 ml of a pretreated and diluted sample spiked or not with As(III) containing 10.0 μl of DDTP (chelating agent) was placed in a sample container. The resulted solution gently shaken few seconds for As-DDTP complex formation and sample container is connected to the extraction vessel via a connector. While opening the embedded valve at the bottom of the sample container and the one in the extraction vessel, the sample solution flows into the extracting solvent with the same flow rate (about 1 ml/min), leading to the successful extraction of As(III) ions that reacted with DDTP into the extracting organic solvent. As soon as the sample solution exits and carries into the extracting solvent, the valve at the bottom of the extraction vessel is turned off. Subsequently, the extraction solvent was collected with a syringe and completely transferred into small conical bottom sample cupe. The small sample cup is transferred to the autosampler. Finally, 20 μl of this extraction solvent using an autosampler was injected into the ETAAS, and the As(III) content was measured based on the temperature program given in Table 1. The total iAs was then measured after the reduction of As(V) to As(III) with sodium thiosulfate and potassium iodide. As(V) was calculated by the difference between the total As and As(III).

Results and Discussion

In this research, a CLLME technique using an extraction solvent lighter than water combined with ETAAS was developed for the extraction and determination of iAs in urine and plasma samples. In order to obtain a high extraction recovery (ER%) and enrichment factor (EF), the effect of different parameters such as kind of extraction solvent and its volume, sample solution flow rate, sample solution volume, sample solution pH, salt addition, amount of chelating agent, and coexisting ions were examined and the optimum conditions were selected. To assess the explained parameters, ER% and EFs have been used. Equations (1) and (2) were used for the calculation of EF and extraction recovery, respectively.

$$EF = \frac{C_{org}}{C_{aq}} \quad (1)$$

where EF, C_{org}, and C_{aq} are enrichment factor, concentration of analyte in the organic phase, and initial concentration of analyte in aqueous sample, respectively.

$$ER\% = \frac{C_{org}}{C_{aq}} \times \frac{V_{org}}{V_{aq}} \times 100 = EF \times \frac{V_{org}}{V_{aq}} \times 100 \quad (2)$$

where ER%, V_{org}, and V_{aq} are extraction recovery, volume of the extraction solvent, and volume of aqueous sample, respectively.

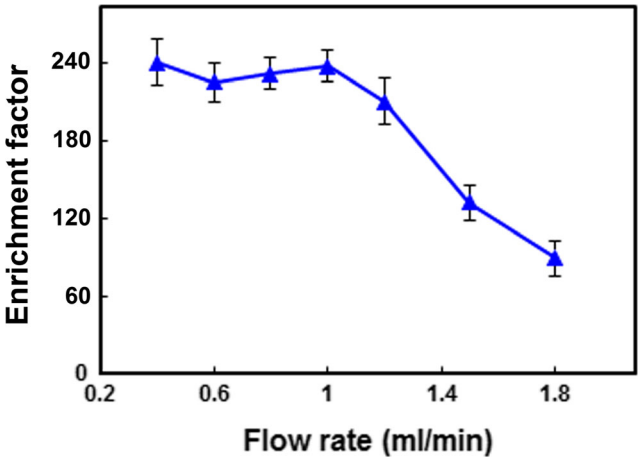

Fig. 2 Effect of sample solution flow rate on the As(III) absorbance from urine or plasma using CLLME procedure. Extraction conditions as in Fig. 1; extraction solvent, toluene

Also, the relative recovery (RR%) of spiked samples was obtained from the following equation:

$$RR\% = \frac{C_{found-Creal}}{C_{added}} \times 100 \quad (3)$$

where C_{found}, C_{real}, and C_{added} are the total concentration of analyte after addition of known amount of standard in real sample, the original concentration of analyte in real sample, and the concentration of known amount of standard which was spiked to the real sample, respectively.

Effect of the Extraction Solvent Type and the Volume

It is one of the most important factors to select a suitable extraction solvent for perform of CLLME method. Extraction solvents are selected based on high extraction capability of the analytes, low solubility in water, lower density than water, low toxicity, and compatible with analytical

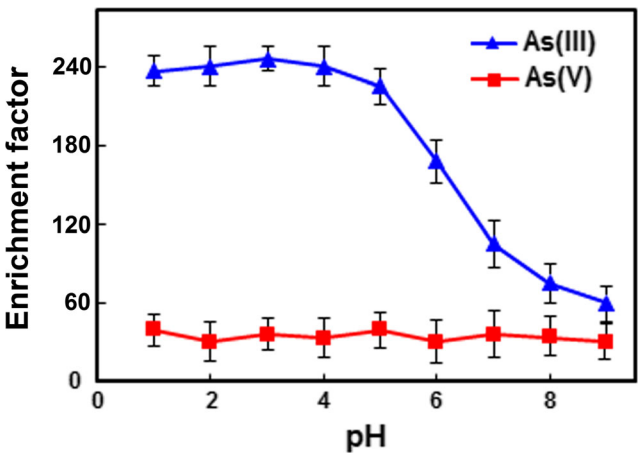

Fig. 3 Effect of sample solution pH on the As(III) absorbance from urine or plasma using CLLME. Extraction conditions are similar to those of Figs. 1 and 2

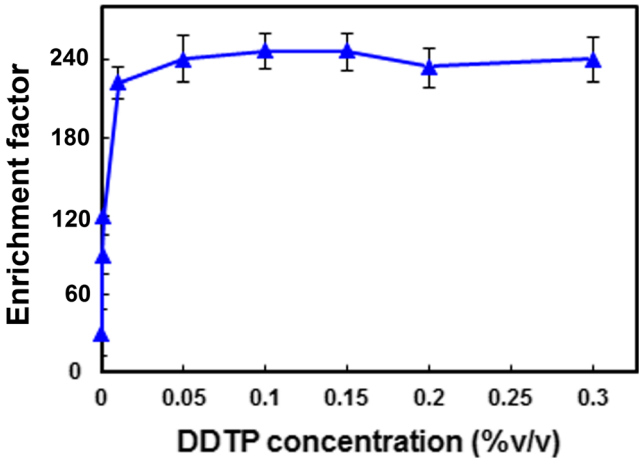

Fig. 4 Effect of the DDTP concentration on the absorbance of As(III) obtained from CLLME. Extraction conditions are similar to those of Figs. 1 and 2

instrument. Therefore, *n*-hexane, 1-octanol, toluene, hexadecane, cyclohexane, and xylene were selected as extraction phase and compared for extraction of As-DDTP complex from aqueous solution. In order to select the best extraction solvent, a series of sample solutions were studied by using 30.0 μl each of the solvent mentioned above. Average absorbances for different extraction solvents are shown in Fig. 1. The results revealed that toluene has the highest absorbance in comparison with the other tested solvents. The reason for this could be that the properties of toluene could be more favorable for the extraction efficiency of target analytes. Therefore, toluene was selected as the extraction solvent.

To examine the effect of the extraction solvent volume on the performance of the CLLME procedure, different volumes of toluene (30–70 μl at 10-μl intervals) were tested. According to Eq. 2, it is clear that by increasing the volume of the extraction solvent, extraction recovery increases. On the other hand, the volume of the remained extraction solvent increases, and as a result, enrichment factor decreases. In many microextraction techniques, EF is more important than extraction recovery and researchers try to keep it as high as possible. In most cases, the lowest volume of the extraction solvent that provides the minimum required amount for analysis is the best choice. The volume less than 30.0 μl, causing difficulties in its injection to ETAAS and encountering systematic errors. Thus, in the following studies, 30.0 μl of toluene was used as an optimal volume of the extraction solvent.

Table 2 Effect of potentially interfering ions on the recovery of 5.0 μg l^{-1} As(III)

Interferent	Interferent/As(III) Ratio	Recovery (%)
Na^+	5000	98.5
K^+	5000	93.0
Li^+	5000	102.5
Ca^{2+}	4000	97.8
Co(II)	1000	99.8
Se(IV)	100	96.0
Sb(III)	100	93.5
Fe(II)	500	101.7
Ni(II)	200	104.2
Zn(II)	100	95.8
Cd(II)	100	91.6
Cu(II)	100	93.4
Cl^-	5000	98.5
SO_4^{2-}	5000	103.0
NO_3^-	5000	101.6

Effect of the Sample Solution Flow Rate

The sample solution flow rate through the extraction solvent is an important factor because it controls the time of analysis and extraction efficiency. The sample solution flow rate must be low enough to accomplish an effectual extraction. On the other hand, it must be highly sufficient not to loss time. The effect of the sample solution flow rate was tested from 0.4 to 1.8 ml min^{-1}. As it is shown in Fig. 2, the flow rates up to 1.0 ml min^{-1} have no effect on analytical signal of As(III) while, at higher speeds, the analytical signal decreased. Thus, a flow rate of 1.0 ml min^{-1} was selected for further studies.

Effect of the Sample Solution Volume

Sample solution volume depends on the nature of the extraction solvent and the type and concentration of sample constituents. The effect of sample solution volume (i.e., 3, 6, 10, 13, 16, and 20 ml) containing a constant amount of As-DDTP, on extraction recovery was investigated. The results showed that an acceptable recovery was observed when sample volumes were increased to 13 ml, which seemed to be the tolerated volume for sample solution. Considering the time of extraction and trace amount of iAs in biological samples, 10 ml was used as the best sample solution volume.

Effect of the Sample Solution pH

pH plays a unique role on the metal–chelate formation and the subsequent extraction. The effect of pH on the complex formation and extraction of As(III) from aqueous samples was studied in the range of 1.0–9.0 by using HNO_3, H_3PO_4, Na_2CO_3, and CH_3COONa. The concentration of As(III) in aqueous sample was 50 ng l^{-1}. The results illustrated in Fig. 3 reveal that the absorbance remained nearly constant in the pH range of 1.0–4.0, and it reduced at higher pH values.

Table 3 Analytical characteristics of CLLME − ETAAS for determination of As(III) in urine and plasma samples

Parameter	Analytical feature	
	Urine	Plasma
Linear range (μg l^{-1})	0.1–50.0	0.15–40.0
r^2	0.9988	0.9955
Limit of detection (μg l^{-1}) (3σ, n = 7)	0.03	0.05
RSD[a] % (Intra-day, n = 7)	2.3	3.5
RSD % (Inter-day, n = 7)	4.0	5.7
Sample solution volume (ml)	10	10
Enrichment factor	240	220
Enhancement factor	212	198

As(III) concentration was 5.0 μg l^{-1} for which RSD was obtained

Also, the effect of pH on the extraction of As(V) species was studied in the abovementioned range. The results showed that the absorbance of As(V) was very low, and the extraction was negligible in this pH range. For further study, a pH of 2.0 was selected.

Effect of the DDTP Concentration

DDTP is a suitable chelating agent for As(III) extraction via the formation of a neutral 3:1 ligand-to-metal complexes species. Therefore, the DDTP concentration was investigated over the range of 0.01 to 0.2% (*v*/*v*). The effect of the DDTP concentration on the analytical signal is shown in Fig. 4. It was found that the analytical signal was increased with the increasing concentration of DDTP from 0.01 to 0.10% (*v*/*v*) and is kept constant upon further increase in concentration. Thus, a DDTP concentration of 0.1% was chosen in subsequent experiments.

Effect of the Coexisting Ions

Most common matrix constituents of real samples such as alkali and alkaline earth elements do not react with DDTP because of its selectivity. The effect of various diverse ions on the determination of 5.0 μg l^{-1} of As(III) was examined separately, as described in the procedure. The criterion for interference of each species was set at ± 5.0% in the analytical signal obtained for a solution containing As(III), without any interfering. The results obtained are given in Table 2. The tolerance limits obtained for interfering metals were so high such that such large amounts of other metal ions could not be found in the biological samples. Therefore, the chances of inferring effects of other metal ions affecting the determination of As(III) in biological samples is expected to be negligible.

Effect of Salt Addition

The effect of salt addition on the extraction of As-DDTP by CLLME method was investigated by adding different amount of NaCl in the range of 0–10% (*w*/*v*). The results obtained from these experiments revealed that the salt addition did not influence the analytical signal significantly for As(III) ions. As a consequence, all the experiments were carried out without salt addition. Also, the results demonstrated the

Table 4 Comparison of CLLME with other extraction methods for determination of As(III) in different samples

Methods	LOD[a] (μg l^{-1})	LR[b] (μg l^{-1})	RSD[c] %	Extraction time (min)	Samples	Reference
DSLLME–ETAAS[d]	0.02	0.08–2	5.3	<3	Environmental water	[4]
SPE–GFAAS[e]	0.00197	0.008–0.26	4.4	30	Water, food and biological samples	[2]
HF-SPME-HPLC–ICP-MS[f]	0.042	0.5–1000	5.1	-	Human urine	[1]
CPE–ETAAS[g]	0.01	0.02–0.35	4.9	<20	Biological and water samples	[33]
MADLLME–ETAAS[h]	0.2	0.5–200	5.3	10	Rice	[16]
CCLLME–ETAAS	0.03–0.05	0.1–50	2.3–3.5	<10	Biological fluids	This work

[a] LOD, limit of detection

[b] LR, linear range

[c] RSD, relative standard deviation

[d] Dispersive-solidification liquid–liquid microextraction and electrothermal atomic absorption spectroscopy

[e] Solid-phase extraction and graphite furnace atomic absorption spectrometry

[f] Hollow fiber-solid phase microextraction coupled with high performance liquid chromatography–inductively coupled plasma mass spectrometry

[g] Cloud point extraction-electrothermal atomic absorption spectrometry

[h] Microwave-assisted dispersive liquid–liquid microextraction and electrothermal atomic absorption spectrometry

Table 5 Determination of As(III) and As(V) in urine and plasma samples, and relative recovery of spiked arsenic in these samples[a]

Samples	Analyte	Added (μg l^{-1})	Found, mean ± SD[b] (n = 3) (μg l^{-1})	Relative recovery (%)
Urine 1 (taken from a 35-year-old male)	As(III)	0	0.60 ± 0.003	–
		0.5	1.12 ± 0.05	104
		1	1.53 ± 0.04	93
	As(V)	0	0.38 ± 0.002	–
		0.5	0.84 ± 0.06	92
		1	1.32 ± 0.1	94
Urine 2 (taken from a 45-year-old male)	As(III)	0	4.25 ± 0.17	–
		5	9.39 ± 0.4	102.8
		10	14.10 ± 0.8	98.5
	As(V)	0	2.60 ± 0.15	–
		5	7.70 ± 0.3	102
		10	12.33 ± 0.8	97.3
Urine 3 (taken from a 32-year-old female)	As(III)	0	0.84 ± 0.04	–
		2	2.90 ± 0.16	103
		4	4.75 ± 0.2	97.7
	As(V)	0	1.20 ± 0.05	–
		2	3.08 ± 0.15	94
		4	5.23 ± 0.3	100.7
Urine 4 (taken from a 28-year-old female)	As(III)	0	7.12 ± 0.4	–
		3	10.22 ± 0.6	103.3
		6	13.04 ± 0.5	98.6
	As(V)	0	4.33 ± 0.18	–
		3	7.40 ± 0.4	102.3
		6	10.12 ± 0.7	96.5
Plasma 1 (taken from a 25-year-old male)	As(III)	0	1.60 ± 0.04	-
		5	6.52 ± 0.2	98.4
		10	11.65 ± 0.6	100.5
	As(V)	0	2.24 ± 0.7	–
		5	7.13 ± 0.4	97.8
		10	12.18 ± 1.0	99.4
Plasma 2 (taken from a 30-year-old female)	As(III)	0	5.63 ± 0.2	–
		2	7.48 ± 0.4	92.5
		4	9.68 ± 0.3	101.2
	As(V)	0	1.52 ± 0.06	–
		2	3.41 ± 0.1	94.5
		4	5.80 ± 0.3	107
NIST SRM-2669	As(III)	1.47 ± 0.10[c]	1.43 ± 0.08	97.2
	As(V)	2.41 ± 0.30[c]	2.44 ± 0.22	101.2

[a] These data are based on the diluted volumes of urine and plasma samples and dilution effect was considered for calculation of them

[b] Standard deviation

[c] Certified values

CLLME method may be in particular suitable for the analysis of some samples with high concentration of salt such as urine and plasma.

Analytical Figures of Merit

To evaluate the usefulness of CLLME–ETAAS for quantitative analysis of As(III) in biological samples, the analytical performance of the proposed method was studied and

validated in terms of linear range (LR), limit of detection (LOD), precision (intra-day and inter-day), EF, and enhancement factor. Table 3 summarizes the analytical characteristics of the optimized method. The intra-day RSDs were determined by five analyses of spiked samples within 1 day, whereas the inter-day precision was examined by analyzing the spiked samples for five consecutive days. Intra- and inter-day precisions of the method based on seven replicate measurements of 5.0 μg l^{-1} of As(III) were in the range of 2.3–3.5 and 4.0–5.7%, respectively. The calibration graph was linear in the range of 0.1–50.0 μg l^{-1} of As(III) with correlation coefficient better than 0.9955. The limit of detection, defined as $D = 3S_b/m$ (where D, S_b, and m are the limit of detection, standard deviation of the blank, and the slope of the calibration graph, respectively), were 0.03 and 0.05 μg l^{-1} in urine and plasma, respectively. High EFs of 240 and 220 were obtained for a 10.0-ml diluted urine and plasma, respectively. The enhancement factor, obtained from the slope ratio of calibration graph after and before extraction, were about 212 and 198 for urine and plasma, respectively.

A comparison between the figures of merit of the proposed CLLME method and some of the published methods for speciation of iAs are summarized in Table 4. It clearly shows that our proposed method has good sensitivity and precision with a wide dynamic linear range. Also, the obtained LODs by this method are better than those obtained by other methods except for SPE. Accordingly, the main advantages of our proposed method include high sensitivity with good precision, rapidity, low consumption of organic solvents, low cost, and simplicity to operation.

Arsenic Determination in Biological Fluids

For further evaluation of the proposed CLLME, As(III) was extracted from both human urine and plasma. All of urine and plasma samples analyzed within 48 h of collection. As(III) and As(V) in all urine and plasma samples were detected at different concentration levels and they were confirmed by spiking As(III) and As(V) into the all samples. The concentration of As(III) and As(V) in the urine and plasma samples is shown in Table 5. Urine and plasma samples were spiked with As(III) and As(V) standards to assess matrix effects. The relative recoveries of As(III) and As(V) from urine and plasma samples at spiking different levels are listed in Table 5. In addition, the accuracy of the proposed CLLME was evaluated by analyzing a standard reference material (SRM) NIST-2669 Inorganic Arsenic Species in Frozen Human Urine from National Institute of Standards and Technology (NIST), with certified As(III) and As(V) content of 1.47 ± 0.10 and 2.41 ± 0.30 μg l^{-1}, respectively. No significant difference was found between the result obtained by employing the proposed method and the certified value (Table 5). These results demonstrated that the matrices of the analyzed urine and plasma samples possess negligible effect on the proposed CLLME followed by ETAAS determination of the iAs.

Conclusions

In this research for the first time, a CLLME using an extraction solvent lighter than water combined with ETAAS has been proposed for the speciation of iAs in biological samples. An especial extraction vessel was designed and found that it can be used for extraction of the iAs into the few microliter organic solvent. In this method, there is a diversity of choices in selecting safe and non-toxic extracting organic solvents such as hydrocarbons and alcohols. Utilization of the CLLME method for the extraction and preconcentration of iAs from urine and plasma was successful. The results of this study demonstrated that the proposed method gives acceptable relative recoveries and repeatabilities for the iAs from biological samples.

As compared with the other sample preparation methods, the analytical procedure offered many advantages such as simplicity, consumes less extraction solvent, ease of operation, high enrichment factor, low detection limit, and relatively short analysis time. Moreover, CLLME is seemed to have huge potential of application for the analysis of other analytes in biological samples, as a beneficial tool in medicine for estimating and personalizing the effective drug dose in patients in clinical situations.

Acknowledgments The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 96422) for the financial support.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent All the participants were informed about the purpose of the study and gave informed consent.

References

1. Chen B, Hu B, He M, Mao X, Zu W (2012) Synthesis of mixed coating with multi-functional groups for in-tube hollow fiber solid phase microextraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in human urine. J Chromatogr A 1227:19–28
2. Hassanpoor S, Khayatian G, Judy Azar AR (2015) Ultra-trace determination of arsenic species in environmental waters, food and biological samples using a modified aluminum oxide nanoparticle sorbent and AAS detection after multivariate optimization. Microchim Acta 182:1957–1965
3. Pu X, Chen B, Hu B (2009) Solvent bar microextraction combined with electrothermal vaporization inductively coupled plasma mass

spectrometry for the speciation of inorganic arsenic in water samples. Spectrochim Acta B 64:679–684
4. Asadollahzadeh M, Tavakoli H, Torab-Mostaedi M, Hosseini G, Hemmati A (2014) Response surface methodology based on central composite design as a chemometric tool for optimization of dispersive-solidification liquid–liquid microextraction for speciation of inorganic arsenic in environmental water samples. Talanta 123:25–31
5. Liang P, Liu R (2007) Speciation analysis of inorganic arsenic in water samples by immobilized nanometer titanium dioxide separation and graphite furnace atomic absorption spectrometric determination. Anal Chim Acta 602:32–36
6. Chen S, Zhan X, Lu D, Liu C, Zhu L (2009) Speciation analysis of inorganic arsenic in natural water by carbon nanofibers separation and inductively coupled plasma mass spectrometry determination. Anal Chim Acta 634:192–196
7. Tang F, Ni Z, Liu Y, Yu Q, Wang Z, Mo R (2015) Arsenic speciation in honeysuckle (Lonicera japonica Thunb.) from China. Biol Trace Elem Res 168:269–275
8. Dai B, Cao M, Fang G, Liu B, Dong X, Pan M, Wang S (2012) Schiff base-chitosan grafted multiwalled carbon nanotubes as a novel solid-phase extraction adsorbent for determination of heavy metal by ICP-MS. J Hazard Mater 219–220:103–110
9. Alqadami AA, Abdalla MA, Alothman ZA, Omer K (2013) Application of solid phase extraction on multiwalled carbon nanotubes of some heavy metal ions to analysis of skin whitening cosmetics using ICP-AES. Int J Environ Res Public Health 10:361–374
10. Huang CZ, Hu B, Jiang ZC (2007) Simultaneous speciation of inorganic arsenic and antimony in natural waters by dimercaptosuccinic acid modified mesoporous titanium dioxide micro-column on-line separation and inductively coupled plasma optical emission spectrometry determination. Spectrochim Acta B 62:454–460
11. Cabon JY, Madec CL (2004) Determination of major antimony species in seawater by continuous flow injection hydride generation atomic absorption spectrometry. Anal Chim Acta 504:209–215
12. Rasmussen RR, Hedegaard RV, Larsen EH, Sloth JJ (2012) Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin. Anal Bioanal Chem 403:2825–2834
13. Tuzen M, Saygi KO, Karaman I, Soylak M (2010) Selective speciation and determination of inorganic arsenic in water, food and biological samples. Food Chem Toxicol 48:41–46
14. Uluozlu OD, Tuzen M, Mendil D, Soylak M (2010) Determination of As(III) and As(V) species in some natural water and food samples by solid-phase extraction on Streptococcus pyogenes immobilized on Sepabeads SP 70 and hydride generation atomic absorption spectrometry. Food Chem Toxicol 48:1393–1398
15. Shamsipur M, Fattahi N, Assadi Y, Sadeghi M, Sharafi K (2014) Speciation of As(III) and As(V) in water samples by graphite furnace atomic absorption spectrometry after solid phase extraction combined with dispersive liquid–liquid microextraction based on the solidification of floating organic drop. Talanta 130:26–32
16. Ahmadi-Jouibari T, Fattahi N (2015) Speciation of inorganic arsenic species and total inorganic arsenic in rice using microwave-assisted dispersive liquid–liquid micro-extraction and electrothermal atomic absorption spectrometry. Food Addit Contam Part A 32:1140–1147
17. Majidi B, Shemirani F (2011) In situ solvent formation microextraction in the presence of ionic liquid for preconcentration and speciation of arsenic in saline samples and total arsenic in biological samples by electrothermal atomic absorption spectrometry. Biol Trace Elem Res 143:579–590
18. Shah AQ, Kazi TG, Baig JA, Arain MB, Afridi HI, Kandhro GA, Wadhwa SK, Kolachi NF (2010) Determination of inorganic arsenic species (As^{3+} and As^{5+}) in muscle tissues of fish species by electrothermal atomic absorption spectrometry (ETAAS). Food Chem 119:840–844
19. Elci L, Divrikly U, Soylak M (2008) Inorganic arsenic speciation in various water samples with GFAAS using coprecipitation. Int J Environ Anal Chem 88:711–723
20. Cheng K, Choi K, Kim J, Sung IH, Chung DS (2013) Sensitive arsenic analysis by carrier-mediated counter-transport single drop microextraction coupled with capillary electrophoresis. Microchem J 106:220–225
21. Jiang H, Hu B, Chen B, Xia L (2009) Hollow fiber liquid phase microextraction combined with electrothermal atomic absorption spectrometry for the speciation of arsenic (III) and arsenic (V) in fresh waters and human hair extracts. Anal Chim Acta 634:15–21
22. Monasterio RP, Wuilloud RG (2010) Ionic liquid as ion-pairing reagent for liquid–liquid microextraction and preconcentration of arsenic species in natural waters followed by ETAAS. J Anal At Spectrom 25:1485–1490
23. Lai G, Chen G, Chen T (2016) Speciation of As^{III} and As^{V} in fruit juices by dispersive liquid–liquid microextraction and hydride generation-atomic fluorescence spectrometry. Food Chem 190:158–163
24. López-García I, Briceño M, Vicente-Martínez Y, Hernández-Córdoba M (2015) Rapid screening of water soluble arsenic species in edible oils using dispersive liquid–liquid microextraction. Food Chem 167:396–401
25. Liu Y, He M, Chen B, Hu B (2015) Simultaneous speciation of inorganic arsenic, selenium and tellurium in environmental water samples by dispersive liquid liquid microextraction combined with electrothermal vaporization inductively coupled plasma mass spectrometry. Talanta 142:213–220
26. Rivas RE, López-García I, Hernández-Córdoba M (2009) Speciation of very low amounts of arsenic and antimony in waters using dispersive liquid–liquid microextraction and electrothermal atomic absorption spectrometry. Spectrochim Acta B 64:329–333
27. Ghambarian M, Khalili-Zanjani MR, Yamini Y, Esrafili A, Yazdanfar N (2010) Preconcentration and speciation of arsenic in water specimens by the combination of solidification of floating drop microextraction and electrothermal atomic absorption spectrometry. Talanta 81:197–201
28. Ahmadi-Jouibari T, Fattahi N, Shamsipur M, Pirsaheb M (2013) Dispersive liquid–liquid microextraction followed byhigh-performance liquid chromatography–ultraviolet detection todetermination of opium alkaloids in human plasma. J Pharm Biomed Anal 85:14–20
29. Pirsaheb M, Fattahi N, Pourhaghighat S, Shamsipur M, Sharafi K (2015) Simultaneous determination of imidacloprid and diazinon in apple and pear samples using sonication and dispersive liquideliquid microextraction. LWT Food Sci Technol 60:825–831
30. Ahmadi-Jouibari T, Fattahi N, Shamsipur M (2014) Rapid extraction and determination of amphetamines in human urinesamples using dispersive liquid–liquid microextraction andsolidification of floating organic drop followed by high performanceliquid chromatography. J Pharm Biomed Anal 95:145–151
31. Bidari A, Jahromi EZ, Assadi Y, Hosseini MRM (2007) Monitoring of selenium in water samples using dispersive liquid–liquid microextraction followed by iridium-modified tube graphite furnace atomic absorption spectrometry. Microchem J 87:6–12
32. Faraji H, Helalizadeh M (2017) Lead quantification in urine samples of athletes by coupling DLLME with UV-vis spectrophotometry. Biol Trace Elem Res 176:258–269
33. Shemirani F, Baghdadi M, Ramezani M (2005) Preconcentration and determination of ultra trace amounts of arsenic(III) and arsenic(V) in tap water and total arsenic in biological samples by cloud point extraction and electrothermal atomic absorption spectrometry. Talanta 65:882–887