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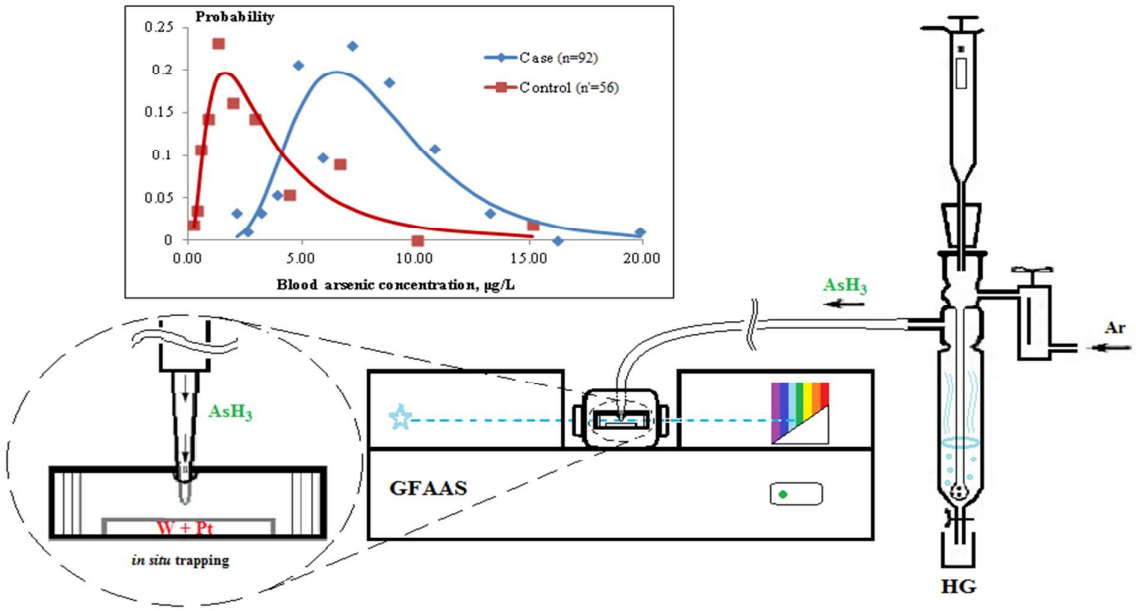
A direct hydride generation GFAAS method of blood arsenic determination and its application for arsenic biomonitoring in infants is presented.



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ARTICLE

Biological monitoring of arsenic pollution based on whole blood arsenic atomic absorption assessment with *in situ* hydride trapping†

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A digestion free blood arsenic assessment method based upon *in situ* trapping of arsenic hydride in coated graphite furnace was developed. Double layer coating of the furnace with Na_2WO_4 – H_2PtCl_6 was used. Arsenic limit of detection (3σ) for whole blood samples was 0.1 μg/L. Dynamic range was 60-1000 pg As. Relative standard deviation for blood samples (n = 3) was 7–13%. Blood arsenic biomonitoring in infants residing in an industrially polluted area was performed using the designed method. Whole blood arsenic determination was performed in 92 subjects of case group and 56 subjects of age-matched control group. For both groups observed blood arsenic distribution was found to be close to lognormal. Lognormalized mean blood arsenic for the case was 5.89 ± 1.31 μg/L; for the controls 1.50 ± 2.26 μg/L. Significant blood arsenic elevation in the case group of the infants under study compared to the controls and previously published data was observed.

Introduction

One of the primal aims of contemporary occupational medicine is a monitoring of chemically exposed territories[1]. Biomonitoring defined as a systematic measurement of biomarkers in human biological media, preferentially blood and urine, for evaluation of uptake and health consequences of chemicals exposure[2,3] is an important part of such studies. Distinct advantage of biomonitoring is integral assessment of exposure from different routes (*e.g. via* gastrointestinal tract, lung and skin)[4] and ability to identify subject subgroups according to exposure level[2]. Arsenic (As) is an inorganic ecotoxicant of top priority[2,5,6]. Hence, arsenic biomonitoring is obligatory, *i.e.* reliable and validated analytical methods for As determination in biological media are required.

Low level, mainly inhalational, arsenic uptake is usually distinctive for chronic occupational exposure according to previously published data[7,8]. On the other hand, considerable natural source (drinking water, foodstuffs) exposure is more typical for endemic regions like West Bengal, Bangladesh and some regions of Mexico[9-12].

Nowadays, inductively coupled plasma mass spectrometry (ICP-MS) is gradually becoming a technique of choice for clinical elemental analysis[13,14]. However, arsenic ICP-MS determination is badly hampered as long as analytical signal of a sole arsenic isotope ($^{75}As^+$) is heavily interferenced with, first of all, $^{35}Cl^{40}Ar^+$ and $^{37}Cl^{38}Ar^+$ signals. Thus, reliable arsenic assessment in biological fluids requires usage of dynamic reaction / collision cell technology[15-17] or double focusing sector field mass spectrometry under high resolution mode $m/\Delta m$ = 10000[18]. Empiric isotope correction is also employed, *e.g.* parallel monitoring of $^{16}O^{35}Cl$ or $^{40}Ar^{37}Cl/^{82}Se/^{83}Kr$ intensities[19]. Noteworthy, robustness of ICP-MS is somewhat limited with nebulization system shortcomings which impede direct injections of non-digested whole blood samples due to nebulizer clogging and memory effects[20].

A conventional method of elemental analysis – graphite furnace atomic absorption spectrometry (GFAAS) still remains amongst important methods of clinical analysis[14,21,22]. The most important advantage of GFAAS compared to ICP-MS is lower costs for instrumentation and materials. Moreover, this technique allows direct determination of trace elements in biological media without preliminary sample digestion[14,21-24]. That helps to eliminate possible systematic errors related to sample preparation[25].

Atomic absorption assessment of arsenic is mainly carried out using hydride generation (HG) technique[26]. Generally total blood sample mineralization as in case of ICP-MS is employed prior to HG-AAS[27,28], arsenic extraction has also been reported[29]. The aim of the present study was to develop a method of digestion free whole blood arsenic assessment with *in situ* trapping and to perform a biomonitoring of infants residing in an industrially polluted area.

Materials and methods

Instrumentation

MGA-915MD (Lumex, Russia) atomic absorption spectrometer with Zeeman polarization modulation background correction[30] was used for measurements. Pyrolitically coated Massman-type graphite furnaces with integrated L'vov platform (Schunk Kohlenstofftechnik, Germany) were employed as atomizers. High frequency lamp (Lumex) was used as radiation source. Arsenic absorption signal was measured at wavelength 193.7 nm as absorption peak area[31]. Arsenic hydride AsH_3 generation was carried out using hydride generator RGP-915 (Lumex). Milli-Q®

Advantage A10 (Millipore, France) system was used for ultrapure water preparation. For sub-boiling distillation of acids distillacid™ BSB-939-IR (Berghof, Germany) system was employed.

Chemicals and reagents

Standard solution 10003-1 As 1000 μg/mL in 2% HNO_3 (High-Purity Standards, USA) was used throughout for calibration. For interference studies multielement standard IV-ICPMS-71A 10.00 μg/mL ea: Ag, Al, As, B, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ho, K, La, Lu, Mg, Mn, Na, Nd, Ni, P, Pb, Pr, Rb, S, Se, Sr, Th, Tl, Tm, U, V, Yb, Zn (Inorganic Ventures™, USA) was employed. Species specific arsenical standards 1000 μg/mL As^{+3} CGAS(3)1-1 and 1000 μg/mL As^{+5} CGAS(5)1-1 (both from Inorganic Ventures™, USA) were used for reduction kinetics studies. Mono- (MMAA) and dimethylarsinic acids (DMAA) were purchased from Supelco® Analytical (Sigma-Aldrich, USA).

All solutions were prepared using Milli-Q® water. For calibration solutions stabilization nitric acid of Suprapure® grade 65% (Merck, Germany) was used. Solutions of organoarsenicals were stabilized with hydrochloric acid. Hydrochloric acid (analytical grade, Lenreaktiv, Russia) was purified at least twice by sub-boiling distillation in BSB-939-IR system prior to use. For arsenic reduction 1% (*w/v*) sodium borohydride $NaBH_4$ solution (Fluka, Sigma-Aldrich, USA) was used. This solution was prepared daily and also contained 0.1% (*w/v*) NaOH (analytical grade, Merck, Germany) for $NaBH_4$ stabilization.

Other reagents and modifiers were: sodium tungstate Na_2WO_4 (Sigma-Aldrich, USA), palladium nitrate for atomic absorption analysis $Pd(NO_3)_2$ (10 g/L, Merck, Germany), Hexachloroplatinum acid hexahydrate $H_2PtCl_6 \cdot 6H_2O$ (Merck, Germany), tetrahydrofuran (Sigma-Aldrich, USA), isoamyl alcohol (Sigma-Aldrich, USA). High purity argon (99.997%, Air Liquide, Russia) was used as shear gas in GFAAS and for AsH_3 transporting into the atomizer.

Reference materials

Seronorm™ Trace Elements Whole Blood L-1 (REF 210105, LOT 1003191) and L-3 (REF 210305, LOT 1003193, Sero AS, Norway) were analyzed for method validation. For quality control purposes reference material analyses were repeated after each 20 real blood samples.

Study population

Totally 148 infants participated in this study. A case group consisted of 92 subjects, residing in a proximity to the chemical weapon utilization unit (Saratov oblast, mean age 8.3 ± 2.4 years). Case group blood sampling was performed during annual medical examination. Age-matched control group was formed from Saint Petersburg inhabitants, who were patients in toxicological policlinics of the Institute of toxicology in 2009-2013 (mean age 10.5 ± 3.2 years). Participants of both groups possessed no major health disorders and could be considered as clinically healthy. Only minor neurological, cardiologic, dermatologic and gastrointestinal disorders were diagnosed in some individuals of this study.

Parents/legal representatives of all patients were informed of the study being performed and signed an informed consent according to legal regulations (the decree of Russian Federation Ministry of Health dated 20th December 2012 number 1177н). Research was carried out according to the ethical regulations.

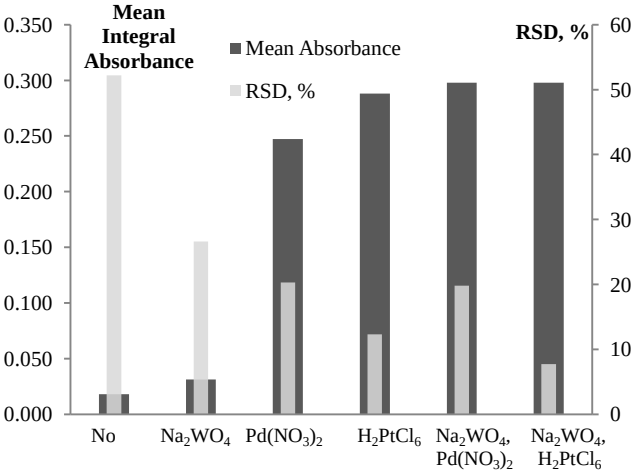

Fig.1 Optimization of graphite furnace coating (2000 pg As, $n = 5$)

Samples

At all stages of investigation a great care was taken to avoid sample contamination. Blood sample collection was performed in clinical conditions in morning hours from cubital vein after fasting overnight by a trained nurse. Vacuum lithium-heparin (1400-1500 U/tube) Vacutest® tubes (Vacutest KIMA, Italy) were used for sampling. Sampled material was frozen at – 20ºC and transported to the laboratory. There samples were thawed at + 4°C and arsenic concentration was determined.

Statistics

Standard program pack Microsoft Excel® 2007 (Microsoft, USA) was employed for statistical calculations. Parametric statistics were used throughout for data evaluation while both case and control groups were large enough (>50). Results distribution type was analyzed using Pearson χ^2 test. Fisher *F*-test was used for dispersion comparison. Mean values were compared using Student's statistics.

Study limitations

Current investigation has several limitations. Subject number in the control group ($n = 56$) was smaller than in the case group ($n = 92$). Also, subjects of the case and control groups lived in different residence environment (small place *vs.* megalopolis). Finally, dietary habits were not considered in the current study.

Results

Method optimization

An important task to solve was to choose an appropriate graphite furnace coating which would provide effective and stable trapping of AsH_3 in the atomizer. Na_2WO_4, $Pd(NO_3)_2$ and H_2PtCl_6 were checked as potential surface modifiers. The results are shown in fig. 1. Optimality criterion was maximal integral absorbance signal for 5 replicates at minimum relative standard deviation (RSD)[31]. Modification procedure was the following: graphite furnace was plunged into 1% (*w/v*) solution of the

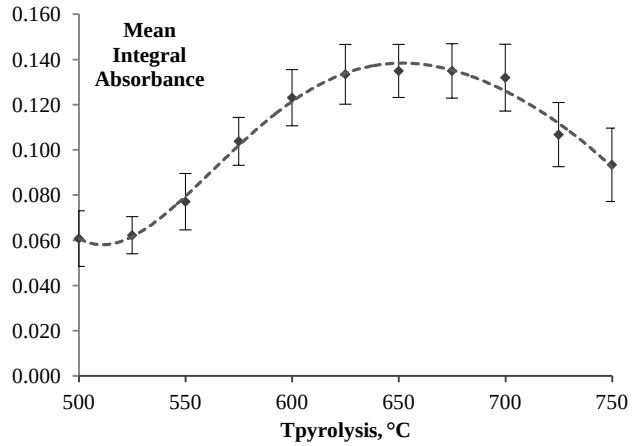

Fig. 2 Dependence of As absorbance on arsine pyrolysis temperature, error bars denote ±SD (1000 pg As, n = 5)

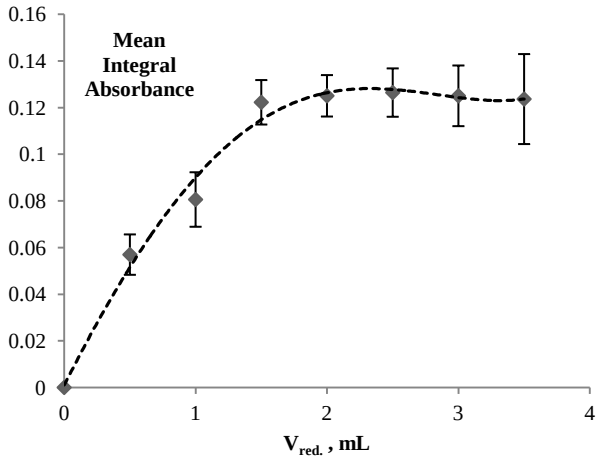

Fig. 3 Dependence of As absorbance on reductant solution quantity, error bars denote ±SD (1000 pg As, n = 5)

corresponding modifier. After that high temperature purge was used for coating formation. The procedure was repeated 6 times. For selection of the optimal coating 200 µL of 10 µg/L As solution (2000 pg) was introduced into the hydride generator. Formed AsH_3 was trapped in the correspondingly coated atomizer and absorbance was measured. Absorbance profiles different furnace coatings used in this study are presented in electronic supplementary information (ESI).

One component modification did not provide stable sorption of AsH_3, hence, double layer modification was used, *i.e.* furnace initially treated with Na_2WO_4 was modified with $Pd(NO_3)_2$ or H_2PtCl_6 respectively. Most effective combination was Na_2WO_4–H_2PtCl_6 (W-Pt). Furnace tungstenation prior to H_2PtCl_6 treatment provided sorption efficacy and stability and also extended furnace lifetime, possibly, due to tungsten carbide microlayer formation protecting graphite against oxidative damage.

Multi-component surface modification was already used for digestion free AAS determination of arsenic and other elements in biological matrices. Freschi *et al.*[33] used W-Ru modification for the determination of As, Bi, Pb, Sb and Se in milk. Liao and Haug[34] performed a systematic study of carbide forming elements (Zr, Nb, Ta, W) and noble metals (Ir, Ir-Mg, Pd-Ir) as potential coating for *in situ* trapping of selenium and tellurium hydrides in

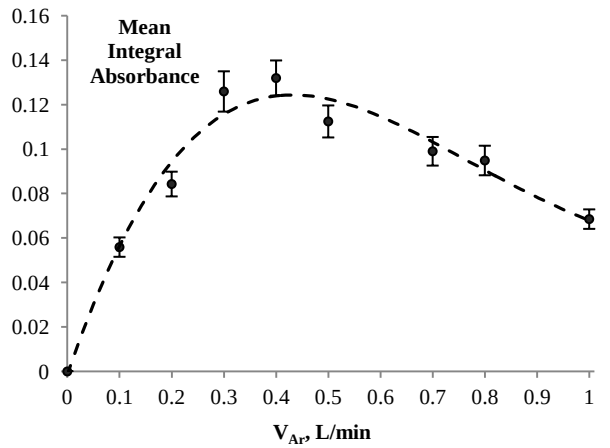

Fig. 4 Dependence of As absorbance on transporting gas flow rate, error bars denote ±SD (1000 pg As, n = 5)

graphite furnace and reported Ir-Mg combination to be the most suitable. For blood and urine selenium assessment Barbosa *et al.*[35] employed W-Rh modification. Haug and Yiping[36] showed the efficacy of Pd-Ir coating for *in situ* trapping of tin hydride. However, most intensively such surface modification was used for mercury determination. Different groups reported usage of W-Pt[22], Zr-Pd[37], Rh-Pd[38], Ir-Au[39] for that purpose.

Since arsine trapping at room temperature resulted in quite narrow linearity range, we used pyrolysis for arsenic trapping. For optimization, 100 µL aliquot of 10 µg/L As was introduced into the hydride generator, system was bubbled with Ar flow for 5 minutes and AsH_3 was transported to the W-Pt modified furnace. After that As integral absorbance was measured. Obtained results are presented in fig. 2. Temperature 650°C being a central zone of obtained plateau was chosen as the most suitable one for *in situ* As trapping. Trapping duration was optimized after reduction condition optimizations (discussed later). Optimized furnace program is shown in table 1.

Table 1 Furnace program optimized for arsenic *in situ* trapping

Stage	t, c	T, °C
Pyrolysis	200	650
Atomization	2	2600
Purge	2	2750
Pause	90	-
Coating	Na_2WO_4, H_2PtCl_6	

Arsenic reduction conditions were optimized under optimum furnace program. Reductant solution was 1% (*w/v*) $NaBH_4$ in 0.1% (*w/v*) NaOH[27,40]. Reduction medium was hydrochloric acid[41], 5 mL HCl was introduced into the hydride generator prior to sample injection. Acid concentration in a wide range (0.1-3M) was found not to influence trapped arsenic absorbance signal. In further studies 0.3M HCl was used as a reaction medium. As for reductant quantity, it was shown (fig. 3) that arsenic analytical signal reached plateau when 1.5 mL of solution was used, whereas injection of more than 2.5 mL resulted in RSD increase.

Transporting gas flow rate was optimized using standard solution of As (100 µL, 10 µg/L As). Absorbance was measured after 3 minute trapping. The results are presented in fig. 4.

Obtained dependence has a maximum near 0.4 L/min, which is in

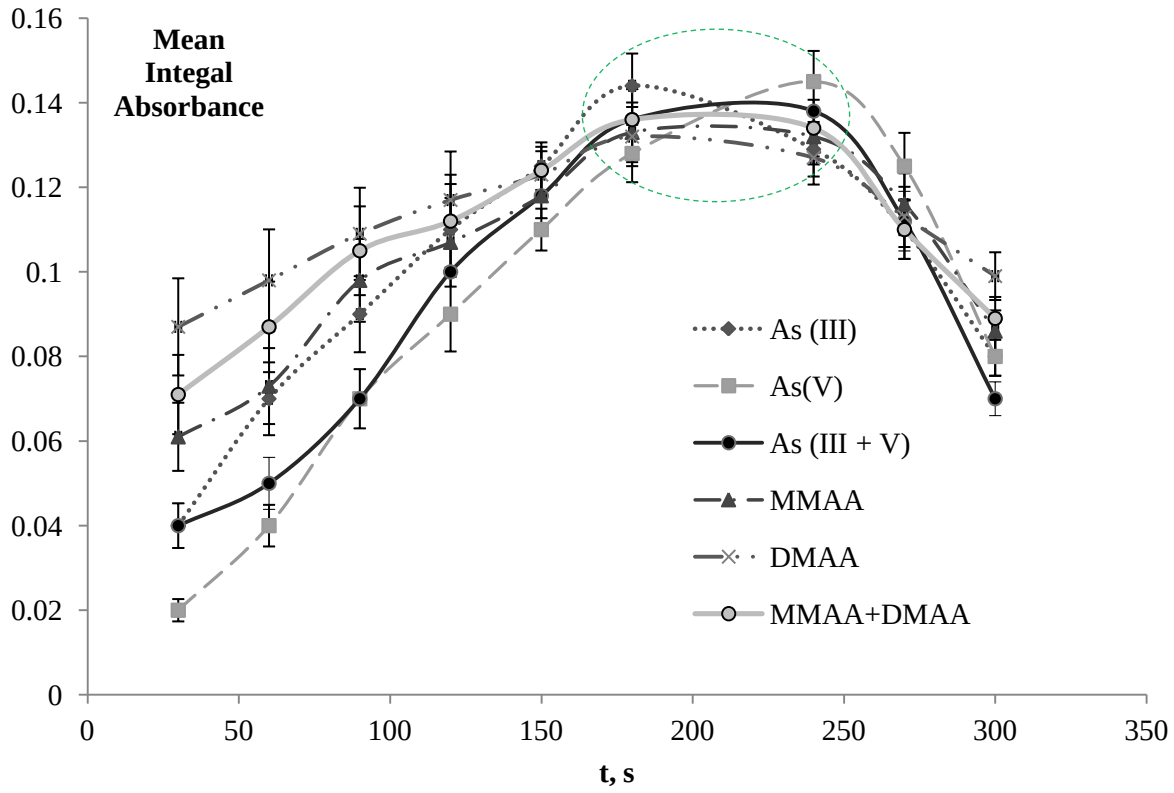

Fig. 5 Time dependence of arsenic absorbance for solutions containing As(III) 10 μg/L; As(V) 10 μg/L; As(III) 5 μg/L + As(V) 5 μg/L; MMAA 10 μg/L; DMAA 10 μg/L; MMAA 5 μg/L + DMAA 5 μg/L (n = 5). Dashed green circle marks the area where all studied arsenic species contributes equally to the analytical signal, error bars denote ±SD

accordance with the studies of Liang *et al*.[42] Afterwards flow rate 0.4 L/min was used in all measurements.

Next step was trapping duration optimization. For that purpose we measured As absorbance varying bubbling duration in the range 30-300 seconds. According to the previously published data, *e.g.* a study[43], reduction rate depends greatly on its spciation, arsenate As(V) being reduced much more slowly than arsenite As(III). That is why we used species specific standards of As(III) and As(V) as well as organoarsenical standards of MMAA and DMAA for reduction kinetics studies. Specifically, 10 μg/L solutions of As(III), As(V), MMAA and DMAA as well as a solutions containing 5 μg/L As(III), 5 μg/L As(V) and 5 μg/L MMAA, 5 μg/L DMAA were used (for all solutions containing organic arsenic species concentrations are given here as calculated for arsenic). Species were reduced and detected using previously chosen conditions. The results are shown in fig. 5.

Contrary to *e.g.* works[27,28,44] only insignificant difference in kinetic profile was observed for As(III) and As(V). The same also referred to MMAA and DMAA. According to our data 1% (*w/v*) $NaBH_4$ solution provided full reduction of both redox As species and two major methylated species of arsenic as well as analyte transport into the graphite furnace within 200 seconds (fig. 5). In other words, no necessity in preliminary As(V) reduction was observed. Noteworthy, hydride generator RGP-915 employed in the current work does not operate in flow injection mode contrary to above mentioned studies[27,28,44], *i.e.* in our case arsenicals reduction took place in a reactor vessel of considerable volume about 8 mL filled. Thus, kinetic limitations for As(V) reduction were virtually leveled, possibly, due to considerable solubility of arsine in water, *i.e.* in our case liquid/gas phase mass carry was a rate limiting stage.

Antifoam reagents were used against considerable foaming when analyzing both real blood samples and reference materials. Addition of 1 mL tetrahydrofuran and 100 μL isoamyl alcohol to the reaction medium was shown to completely remove undesirable foam formation when 1/10 (*v/v*) blood was analyzed. Arsine reduction parameters are presented in Table 2.

Table 2 AsH_3 reduction and transporting optimum parameters

Parameter	Value
Trapping duration, seconds	200
Flow rate, L/min	0.4
Aliquot volume, μL	100 – 3000
Dilution factor	1/10 (*v/v*)
Media	5 mL 0.3 M HCl
Reductant	2.5 mL 1% $NaBH_4$ in 0.1% NaOH
Antifoam	1 mL THF[a], 100 μL IA[b]

[a] THF – tetrahydrofuran; [b] IA – isoamyl alcohol

Thus, a new digestion free method of whole blood arsenic determination by graphite furnace atomic absorption spectrometry with *in situ* hydride trapping was developed.

Procedure

Arsenic quantification was performed using external calibration. Aqueous solutions of As were used throughout for calibration. Integral absorbance signals for standards were measured after *in situ* trapping of arsine in concordance to the designed method for the real blood samples. For blood samples: an aliquot (100-3000 µL) of 1/10 (*v/v*) diluted whole blood was introduced into hydride generator RGP-915 containing 5 mL 0.3 M HCl, reductant solution (2.5 mL 1% $NaBH_4$ in 0.1% NaOH) was added and AsH_3 formed was transported to W-Pt coated graphite furnace with 0.4 mL/min argon flow (Table 2). Arsine trapping was performed for 200 s at 650°C. Arsenic integral absorbance signal was measured according to the furnace program presented in Table 1.

Analytical figures of merit

Reference materials Seronorm™ were analyzed for method validation. Measured concentrations met target values – Table 3.

Table 3 Analysis of reference materials Seronorm™ Trace Elements Whole Blood

Level	Measured concentration, µg/L (n = 6, P = 0.95)	Target value, µg/L	Recovery, %
1	4.9 ± 0.3	5.7 ± 3.0	86.0
3	32.7 ± 1.7	30.9 ± 3.8	105.8

Standard addition technique was used for additional validation (spike recoveries were in the range 91-97%). Acquired results for the developed method were also comparable to results of sector field inductively coupled plasma mass spectrometry obtained using a method described in the work[18] (data not shown). Result precision (RSD) for real blood samples (n = 3) were in the range 7-13%.

Limit of detection (LoD), estimated as 3σ of blank solution (n = 10) was 30 pg which corresponds to 0.1 µg/L when calculated for 3000 µL of 1/10 (*v/v*) diluted blood. Conventional matrix effects for real blood samples (*e.g.* chloride and other halides, high background absorption) were absent due to the separation of the analyte from the matrix of the blood. However, for blood samples co-reduction of other hydride forming element, first of all, selenium and lead, was observed which led to shortage of linearity range of the method. Linearity range for standard solutions was 60-2000 pg As. Co-reduction of selenium and lead led to the concurrent occupation of AsH_3 sorption sites and decreased the linearity range to 1200-1500 pg As. Such matrix influences were proven by analyzing a standard solution containing 10 µg/L As, Se and Pb and also by analyzing reference material Seronorm™ Trace Elements Whole Blood L-3, containing 250 ± 50 µg/L Se and 638 ± 22 µg/L Pb along with As 32.7 ± 1.7 µg/L. Accordingly, the dynamic range of the method was set to 60-1000 pg As.

Real blood samples analysis

Using the developed method 92 samples of the case group and 56 samples of the control group were analyzed for arsenic content. Each sample was analyzed at least 3 times and mean value was calculated. A result was considered acceptable when RSD (n = 3) did not exceed 15%. Otherwise measurements were repeated after exclusion of inacceptable precision source and fulfilling quality assurance protocols. The results are presented in Table 4.

Table 4 Whole blood arsenic concentration for the case and control groups

Parameter	Value, µg/L	
	Case	Control
Mean	6.36	1.83
Median	6.25	1.10
SD	2.46	1.75
Minimum	1.81	0.25
5th percentile	2.96	0.40
25th percentile	4.70	0.80
50th percentile	6.25	1.10
75th percentile	7.70	2.30
95th percentile	10.38	5.03
Maximum	16.3	10.3

Discussion

Developed method of blood arsenic determination

The developed method allowed reliable assessment of blood arsenic reference concentration lower limit as well as sub-toxic and toxic levels of this element. Acquired LoD (0.1 µg/L) was lower than one obtained by ICP-MS with a dynamic reaction cell after a preliminary microwave digestion. D'Ilio *et al.*[15] reported the latter to be 2.03 µg/L whereas in further studies by this group[45] LoD value of 0.7 µg/L was presented. LoD was also comparable to ICP-MS method with 10% tetramethylammonium hydroxide with final sample dilution 1/50 (*v/v*) being 0.08 µg/L according to the work of Nunes *et al.*[21] Bazzi *et al.*[16] used acid digestion with a mixture of concentrated HNO_3 and 30% H_2O_2. They reported 0.20 µg/L as arsenic LoD for ICP-MS with a dynamic reaction cell. Heitland and Köster[46] obtained limit of quantification for the digestion free whole blood arsenic determination after 1/10 (*v/v*) sample dilution with 0.1% Triton X-100 and 0.5% (*v/v*) NH_3 by ICP-MS with a collision cell (He/H_2) being 0.1 µg/L.

In the study of de Souza *et al.*[28] authors determined blood arsenic by AAS with flow injection hydride generation and *in situ* trapping of AsH_3 in a tungsten atomizer modified with 200 µg rhodium. Samples were digested with a mixture of 20% HNO_3 and 30% H_2O_2. 30 minute incubation with L-cysteine was used for preliminary reduction of As(V) to As(III). LoD was 0.11 µg/L. Afridi *et al.*[5] used GFAAS for blood arsenic assessment. Blood samples were digested in a microwave system with concentrated HNO_3 and 30% H_2O_2. As was determined with conventional $Pd(NO_3)_2$+$Mg(NO_3)_2$ mixture[47] as a modifier. LoD was apparently presented for pure solutions being 15.9 pg/g which corresponds to about 0.38 µg/L for blood samples. For similar method reported in a study[48] As LoD was 0.22 µg/L. Campillo *et al.*[49] performed digestion free GFAAS determination of blood arsenic after 1/4 (*v/v*) with 0.1% (*w/v*) Triton X-100. For matrix interferences coping Zeeman correction system and co-injection of a mixture containing 15% H_2O_2, 0.65% HNO_3 and 0.5% Ni (all *w/v*) were used. Reported LoD was 2 µg/L.

It might be concluded that analytical method developed in the current study has comparable limits of detection to ICP-MS

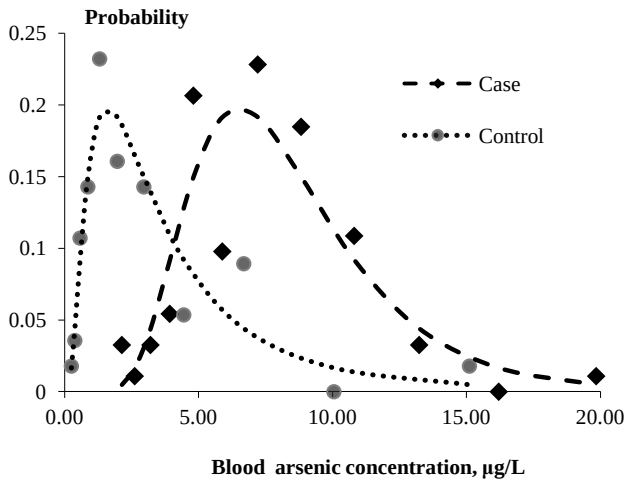

Fig. 6 Blood arsenic concentration for case and control subjects. Dashed lines show theoretical lognormal distribution corresponding to experimentally found mean and SD parameters

methods and in a majority of cases excels both AAS and ICP-MS methods containing preliminary blood sample digestion.

Biomonitoring results

Blood arsenic concentration distributions for both case and control groups were far from normal type according to χ^2 Pearson test (case group $\chi^2 = 5.60 \cdot 10^5 >> \chi^2_{crit} = 122.94$ at confidence level $\beta = 0.01$; control $\chi^2 = 7.97 \cdot 10^7 >> \chi^2_{crit} = 79.87$, $\beta = 0.01$). Notably, case group distribution could be normalized after exclusion of the maximum value: $\chi^2 = 72.95 < \chi^2_{crit} = 94.44$ ($\beta = 0.3$). However, analogous exclusion did not normalize the result distribution for the control group. Further distribution analysis showed that for both groups of the current study result distributions were close to lognormal (case group $\chi^2 = 36.23 < \chi^2_{crit} = 95.48$, $\beta = 0.3$; control $\chi^2 = 9.57 < \chi^2_{crit} = 57.88$, $\beta = 0.3$). Both distributions are presented in fig. 6.

Parameters of acquired distributions were: mean $\ln C_{As}^{case} = 1.77$, SD $\ln C_{As}^{case} = 0.40$; mean $\ln C_{As}^{control} = 0.27$, SD $\ln C_{As}^{case} = 0.81$ which corresponds to $C_{As}^{case} = 5.89 \pm 1.31$ µg/L; $C_{As}^{control} = 1.50 \pm 2.26$ µg/L. Fisher F-test $\ln C_{As}$ deviation comparison for case and control showed that result dispersion differed significantly: $F = 4.05 >> F_{crit} = 1.41$ ($\beta = 0.05$). Consequently, relative homogeneity of arsenic exposure in the case group compared to the controls could be supposed. That seems quite logical as subjects of the case group are residing in a small place (Saratov oblast, ~ 6000 inhabitants) having much closer living and dietary conditions than the controls which were mainly selected amongst megalopolis residents (St. Petersburg, ~ 5 million inhabitants). Since our groups had significantly different deviations and different number of subjects, for mean $\ln C_{As}$ comparison Welch t-test was employed. Calculated test value was: $t_{Welch} = 12.86 >> t_{crit} = 3.45$ at confidence level $\beta = 0.001$ and degrees of freedom $\nu = 72$, *i.e.* difference between case and control blood arsenic concentration was statistically significant.

Comparison of the acquired data to the literature showed that both mean and median values of blood arsenic concentration for the case group were significantly higher than previously published data for unexposed population. For instance, blood arsenic reference values for unoccupied subjects presented in the study[10] were in the range 0.3-2 µg/L. Bazzi *et al.*[16] reported blood arsenic distribution for South Africa school children (n = 48) which was quite similar to ours. Moreover, our control group results are quite coincident to those of South Africa – 1.83±1.75 *vs* 1.53 ± 0.53 µg/L (mean ± SD). Blood arsenic concentration range of 0.1-3.2 µg/L was reported for general population (n = 1554) of Brasilia[21]. For occupationally unexposed population of Germany (n = 130) the range was 0.13-4.2 µg/L, while 5^{th}-95^{th} percentile range was only 0.16-2.3 µg/L according to Heitland and Köster[46]. Khlifi *et al.*[28] presented values for healthy inhabitants (n = 350) of industrially polluted area in South Tunisia being <0.03-18.15 µg/L. Nevertheless, their mean value 1.56 ± 2.49 µg/L was significantly lower than for our case group yet comparable to the controls in the current study. Noteworthy, for subgroup of pesticide exposed farmer and subjects exposed to wood fumes reported values were 3.57 ± 4.97 and 3.07 ± 7.38 µg/L respectively[28].

As a resume, a considerable arsenic pollution could be concluded for the industrially exposed area under study. Main arsenic exposure source is possibly related to air as well as drinking water pollution due to chemical emissions from nearby utilization plant. Arsenic uptake with foodstuffs seems unlikely as nowadays local food consumption is quite rare for Russian population. However, a comprehensive chemical monitoring of the territory is required for accurate exposure evaluation and risk assessment. Anyway, more insight is recommended for occupational safety, ecological monitoring and control of hazardous emissions in the area.

Conclusion

A method for digestion free whole blood arsenic assessment based on *in situ* trapping of arsenic hydride in a coated graphite furnace was developed. Current method is rapid and robust as it does not require a complicated preliminary sample preparation or analyte separation / pre-concentration and has improved limits of detection compared to previously reported atomic absorption methods. Moreover, limits of detection of the current method are even comparable to inductively coupled plasma mass spectrometry methods of whole blood arsenic assessment with preliminary sample digestion. The method was shown to be sensitive enough for background blood arsenic level assessment. Developed method was used for arsenic biomonitoring in infants living in the industrially polluted area. Significant whole blood arsenic elevation compared to the age-matched control and previously published data for occupationally unexposed population was established.

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Notes and references

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