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Author(s)	Nguyen, Thi Anh Huong; Pham, Hung Viet; Peter, C. Hauser et al.
Citation	Annual Report of FY 2007, The Core University Program between Japan Society for the Promotion of Science (JSPS) and Vietnamese Academy of Science and Technology (VAST). 2008, p. 3-10
Version Type	VoR
URL	https://hdl.handle.net/11094/13199
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A PORTABLE CAPILLARY ELECTROPHORESIS SYSTEM FOR INORGANIC ARSENIC DETERMINATION

Nguyen Thi Anh Huong¹, Pavel Kubáň^{2,3}, Pham Hung Viet¹, Peter C. Hauser²

¹*Research Centre for Environmental Technology and Sustainable Development (CETASD),*

Hanoi University of Science, 334 Nguyen Trai Street, Hanoi, Vietnam

²*University of Basel, Department of Chemistry, Spitalstrasse 51, 4004 Basel, Switzerland*

³*Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic,*

Veve i 97,61142 Brno, Czech Republic

Abbreviations:

C⁴D: capacitively-coupled contactless conductivity detection

CAPS: 3-(cyclohexylamino)-1-propanesulfonic acid

CHES: 2-(cyclohexylamino)ethanesulfonic acid

MES: 2-morpholinoethanesulfonic acid

MOPS: 3-morpholinopropanesulfonic acid

TAPS: N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid

Arg: L-arginine

CTAB: cetyltrimethylammonium bromide

Keywords: Inorganic arsenic ion, Capillary electrophoresis, Contactless conductivity detection, Portable instruments

Abstract

A portable capillary electrophoresis instrument with capacitively coupled contactless conductivity detection (CE-C⁴D) was developed and optimized for field measurements of inorganic arsenic species in environmental samples. The determination of As(III) and As(V), as inorganic arsenite and arsenate, was investigated. It was found necessary to determine the two inorganic arsenic species separately employing two different electrolyte systems. Electrolyte solutions consisting of 50mM CAPS/2mM Arg (pH 9.0) and of 45mM acetic acid (pH 3.2) were used for arsenic(III) and arsenic(V) determinations, respectively. Detection limits of 0.29µM and 0.15µM were achieved for As(III) and As(V) respectively by using large volume injection to maximize the sensitivity. The analysis of contaminated tubewell water samples from Vietnam is demonstrated.

1. Introduction

Arsenic contamination in Vietnam has been much concerned by the Government and scientists [3, 4, 5]. This should be extremely serious when the people are still using tubewell water for cooking and domestic activities which are not checked and treated with arsenic and other toxic substances. Among arsenic components, the two main forms of inorganic arsenic, arsenite (As(III)) and arsenate (As(V)), have usually occurred in groundwater. Beside the advanced methods used for arsenic analysis in laboratories such as Atomic Absorption Spectrometry (AAS), Atomic Fluorescent Spectrometry (AFS), Electrochemical methods (Voltammetry), UV-Vis, Induced Couple Plasma - Mass Spectrometry (ICP-MS), Ion Chromatography - Induced Couple Plasma - Mass Spectrometry (IC-ICP-MS), etc. other methods have been developed in purpose of field measurements such as test-kits (based on the reactions between arsine (AsH₃) and mercury bromide creating yellow and tested with standard colour chain) and biosensors using bacterial markers [13].

In this research, in making small contributions to the mitigation of arsenic exposure in contaminated groundwater in domestic activities, a latest research on a portable capillary electrophoresis

instrument with capacitively coupled contactless conductivity detection has been introduced. The method has produced the preliminary results in analysis of inorganic arsenic species reaching the WHO guideline (50 µg/L). The system and the optimization procedures for field measurement of inorganic arsenic species have been illustrated.

2 Materials and Methods

2.1 Instrumentation

Separations were carried out on an instrument, which was built in-house [6, 7, 8] and is based on a high voltage power supply with interchangeable polarity (CZE 2000R) from Start Spellman (Pulborough, UK). The contactless conductivity detector consists of two tubular electrodes of 4mm length separated by a gap of 1mm and a Faradaic shield [11, 12]. Cell excitation was carried out with a sine-wave with a frequency of 200 kHz and a peak-to-peak amplitude of 300V. The resulting current signal was amplified, rectified and low pass filtered with a circuitry described elsewhere [12] before passing to a MacLab/4e data acquisition system (AD Instruments, Castle Hill, Australia) for recording of the electropherograms. All electropherograms were inverted for presentation of the peaks in the normal orientation. Detection limits are reported as the concentrations giving peak heights corresponding to three times the baseline noise. pH-measurements were carried out with a model 744 pH-meter from Metrohm (Herisau, Switzerland).

2.2 Reagents and methods

All chemicals were of analytical reagent grade and were obtained from Fluka (Buchs, Switzerland). Stock solutions (10mM) of As(III) and As(V) were prepared from sodium arsenite (Fluka, Buchs, Switzerland) and di-sodium hydrogen arsenate (Merck, Darmstadt, Germany), respectively. Stock solutions of inorganic anions (10mM) were prepared from the corresponding sodium or potassium salts (Fluka or Merck). All multi-ion standard and calibration solutions were prepared from these stock solutions. All solutions were prepared using deionised water, degassed by ultrasonication and filtered through 0.2 µm nylon filters before use. Fused-silica capillaries of 50µm i.d. and 375µm o.d. were used for performing the electrophoretic separations. These were purchased from Polymicro Technologies (Phoenix, AZ, USA) and were preconditioned with a 0.1M sodium hydroxide solution before flushing with water followed by flushing with the running buffer. The total length of the separation capillaries was 50cm (L = 43cm eff.) and 75cm (L=68cm eff.). Sample injection was carried out in the hydrodynamic mode by elevating one end of the separation capillary to a given height for a specific time.

3 Results and discussion

3.1 Optimization of analytical procedure for the determination of As(III)

pKa₁ of arsenous acid is 9.2 leading to the pH value of electrolyte solutions using for As(III) analysis which should be around or above 9.0 in order to achieve good results. Among some basics usually used for electrophoretic separation, especially for conductivity detection, selected L-arginine has pKa value of 12.5. Acidic-counter ions for electrolyte solutions shall be investigated based on the components of MES, CHES, MOPS, TAPS, CAPS. pH value of the solution will be adjusted in accordance with the varying ratio of L-arginine and acidic components. The investigation of the different pH values ranging from 8.0 to 10.0 based on the electrolyte solutions shown that if the pH values are lower than 9.0, the peak of As(III) is small, if the pH values are higher than 9.0, the peak of As(III) is bigger but the baseline-noise signal is much more caused by the increased conductivity of the electrolyte solutions as well as the separation between As(III) and carbonate will decrease since the peak of As(III) shifted to the peak of carbonate, which should be more affected by using large volume injection to increase the sensitivity for As(III) analysis. Thus, based on the comparison of signal-to-noise ratio, the optimal pH value for As(III) analysis will reach 9.0. The comparison results of electrolyte solutions at pH 9.0 are demonstrated in Figure 1.

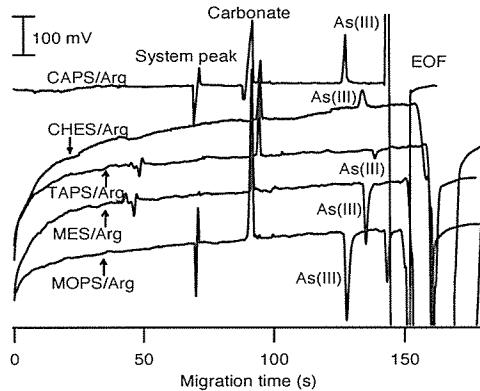


Figure 1. Electropherograms for a standard solution of 100 μM As(III) in different electrolyte solutions at pH 9.0 (30 μM CTAB was added to all buffers for EOF modification). Sample injection: hydrodynamic, 20 cm for 10 s. Detection parameters: 300 Vp-p, 200 kHz. Separation potential: -20 kV. Capillary: fused-silica, 50 μm i.d., $L = 50\text{ cm}$ ($L_{\text{eff}} = 43\text{ cm}$).

From the results in Figure 1 and the calculation of signal-to-noise ratio by comparing the peak heights with the level of noise present on the baseline show that the best electrolyte solution for As(III) analysis is CAPS/Arg, although the peak height of As(III) is not the highest. Moreover, this electrolyte solution has the lowest conductivity among the investigated solutions. Thus, CAPS/Arg is chosen for As(III) electrophoretic separation.

To enhance the sensitivity for the As(III) analysis, large volume injections were used and the capillary length was increased up to 75cm. Based on the selected electrolyte solution, the time for sample injection on the principle of hydrodynamic mode was investigated in 10 to 120s. The 20cm height of sample injection remained unchanged in all experiments. Longer sample injection time will obtain better peak but the solution between peaks will decrease, especially when conducting the analysis on the real samples with the high content of other anions. Thus, the sample injection time of 60s was chosen for ensuring the optimal solution and sensitivity.

In conclusion, the optimal conditions for As(III) based on CE are: 50mM CAPS/2mM Arg at pH 9.0 (30 μM CTAB was added to all buffers for EOF modification). Sample injection: hydrodynamic, 20cm for 60s. Detection parameters: 300Vp-p, 200kHz. Separation potential: -20kV. Capillary: fused-silica, 50 μm i.d., $L = 75\text{cm}$ ($L_{\text{eff}} = 68\text{cm}$).

Based on these optimal conditions, the standard calibration with the As(III) concentration ranging from 1 to 100 μM was gained. The calculated limit of detection (LOD) based on the peak height and signal-to-noise ratios for three times is 0.29 μM with the correlation coefficient (r^2) is 0.9975. The repeatability of peak areas was determined and calculated based on different sample injection of standard solutions in three times and the obtained RSD is ranging from 0.1% to 6.8% with the As(III) concentration of 1 to 100 μM .

Groundwater samples were taken from the high risk areas of arsenic contamination in Vietnam [1, 2, 3, 13] (samples VN1 and VN2 collected from Hanam Province and samples VN3 to VN5 from Thanh Tri, Hanoi). These samples were directly pumped from tube wells and kept in plastic bottles (cleaned in turn by detergent, deionised water, 0.1M nitric acid, deionised water and water samples in three times). These samples were preserved in 4°C before and after being delivered to Switzerland for further analysis. Then, the samples were filtered through 0.20 μm nylon syringe filter before being analysed by CE. Because As(III) is not endurable and easily oxidised by oxygen to develop into As(V) and almost groundwater in Vietnam contains high iron concentration leading

to the arsenic adsorption or/and precipitation to iron(hydr)oxides so As(III) was not found in all samples taken from Vietnam. However, these samples were added As(III) to prove the analysis potential of small As(III) concentration in real sample background from Vietnam. Figure 2 illustrates one of the electropherograms for the analysis of spiked-samples from Vietnam by using the above mentioned optimal conditions.

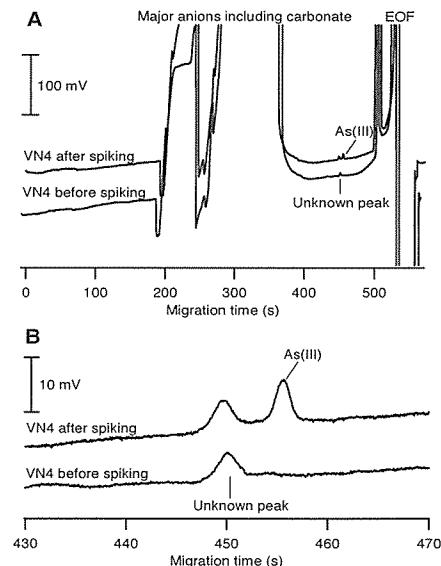


Figure 2. A) Determination of As(III) in ground water sample (VN4) from Vietnam. The spiked concentration of As(III) is $1.5 \mu\text{M}$. Electrolyte solution: CAPS 50 mM / Arg 2 mM, 30 μM CTAB, pH 9.0. Sample injection: hydrodynamic, 20 cm for 60 s. Detection parameters: 300 Vp-p, 200 kHz. Separation potential: -20 kV. Capillary: fused-silica, 50 μm i.d., $L = 75 \text{ cm}$ ($L = 68 \text{ cm}$)
B) Enlarged sections of the electropherograms.

From the electropherograms, it is evident that the arsenic analysis with small concentrations in complex sample backgrounds with other anion concentrations is much higher than arsenic concentrations. Moreover, in such complex sample backgrounds, arsenic peaks are completely separated and not influenced by much higher peaks as demonstrated in the enlarged sections of electropherogram (figure B). Unknown-peak close to arsenic peak in the figure is observed in groundwater sample background not only in Vietnam but also in Basel (Switzerland) such as Rhein River, tap water, bottle water although the peaks are not determined.

The recovery values were investigated based on the five tubewell water samples from Vietnam and three water samples (tap water, bottle water and Rhein River water) from Basel when adding $1.5\mu\text{M}$ As(III). The obtained recovery for spiked-samples is ranging from 88 to 120%. The results show that it is possible to determine As(III) reliably in real samples at this level.

3.2 Optimization of analytical procedure for the determination of As(V)

Primarily, the determination of As(III) and As(V) with the same electrolyte solutions and in the same electropherograms was conducted. But the results shown that As(V) peaks, although rather sensitive when being analysed at pH 9.0 higher than pKa 2.2 and 7.1, were much affected by other anions in real samples, especially phosphate and carbonate since they had the same migration time with As(V) while their concentrations were much higher than As(V). Thus, As(V) analysis will be investigated based on the electrolyte solution with lower pH. In addition, the separation between As(V) and phosphate need to be investigated since they have the same pKa values and characteristics. Based on the preliminary investigation of histidine/ acetic acid buffer solutions with

various pH as well as the use of modelling software PeakMaster 5.1 [14] for electrophoretic process, it is proved that the decrease in pH, or in other words, only by using acid acetic will bring about better results. The acetic acid concentrations ranging from 10 to 500mM which are used for As(V) analysis and the separation between As(V) and phosphate are illustrated in Figure 3. This shown that the higher acetic acid concentrations are, the better the separation between As(V) and phosphate is, however, the peak signals of As(V) and phosphate are declined. The selected optimal concentrations of acetic acid are 45mM.

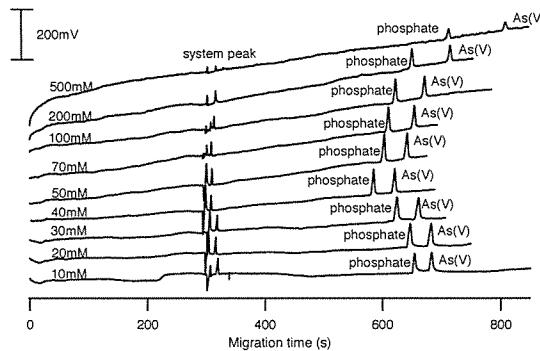


Figure 3. Optimization of the acetic acid concentration for As(V) determination and separation between As(V) and phosphate. Sample injection: hydrodynamic, 20 cm for 60 s. Other conditions as for Figure 2

Other conditions such as the capillary length, sample injection time bring about the similar results as in As(III) analysis. The six point standard calibration with As(V) concentration ranging from 0.5 to 100 μ M was conducted with the correlation coefficient (r^2) of 0.9998. LOD calculated based on 3 S/N criterion and the peak height is 0.15 μ M. The RSD values of peak areas in the concentration range from 1 to 100 μ M were between 1.3 to 2.3%.

Sample analysis of five groundwater samples from Vietnam was conducted. Arsenic concentrations were found in three out of these samples, the results were illustrated in Figure 4. Like the case of As(III), spiked samples in the remaining samples were analyzed. The recovery values when adding 1.0 μ M As(V) into the spiked-samples is from 93 to 110%. This good result proves that the As(V) analysis method is absolutely reliable.

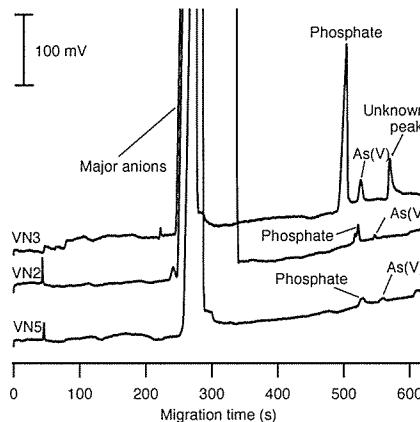


Figure 4. Determination of As(V) in ground water samples from different places in Vietnam. Electrolyte solution: 45 mM acetic acid, pH 3.2. Sample injection: hydrodynamic, 20 cm for 60 s. Other conditions as for Figure 2.

The analysis results of As(V) concentrations in three groundwater samples are 0.16 ± 0.01 , 1.81 ± 0.06 and 0.18 ± 0.01 in correspond with samples VN2, VN3 and VN5. The element used for quantitative analysis is based on the peak areas in principle of standard addition so the obtained results are completely trustworthy.

3.3 Optimization of CE-C⁴D portable system for arsenic determination

The system is constructed with the dimension $310 \times 220 \times 260$ mm, made of poly(methylmethacrylate) (PMMA) with the total weight of 11,2 kg. Detectors (dimension $70 \times 205 \times 160$ mm) and voltage power supply are attached with the system (inside or behind), which is very convenient for the portable use. The power supply of the system is 12V rechargeable battery. The 12V battery is a NP 3.2-12 acid-lead battery (Yuasa Battery UK Ltd., Swindon, UK) and has a capacity of 3.2Ah. Two high voltage supplies are used in the portable CE instrument. The DX150 and the DX150N (EMCO, Sutter Creek, CA, USA) modules are capable to produce positive and negative high voltages, respectively, in the range from 2 to 15kV. The high voltage units are controlled with a multi-turn potentiometer. The polarity of the applied separation voltage is set by a switch but also requires manual replugging of the high voltage lead into the appropriate connector in the back of the operator's compartment. The instrument electronics are powered by batteries and the high voltage modules are capable of delivering up to 15kV at both polarities for more than one working day.

The portable system will be used for arsenic analysis in the optimised conditions together with the standard system for testing the system operations. In this case, the standard calibration build-up and As(V) analysis in groundwater samples from Vietnam was conducted. The applied conditions are acetic acid 45mM and samples are injected in principle of hydrodynamic mode at 20cm in 60s. However, due to the nature of the portable system, some parameters will be changed and optimised for single use. The capillary will be shortened to 60cm (53cm effective length) in comparison with 75cm since the voltage power supply is only 15kV compared to 20kV as the standard system. To achieve sensitivity as much as in standard system with shorter capillary the resistor was changed from $560 \text{ k}\Omega$ to $2.2 \text{ M}\Omega$.

Five point standard calibrations were carried out similarly with the standard instrument in concentration range of 1 to $100 \mu\text{M}$ and the correlation coefficient (r^2) of 0.9997. LOD and LOQ were calculated based on 3 S/N and 10 S/N criterion and were estimated to $0.20 \mu\text{M}$ and 0.66 , respectively. RSD values of peak areas in the concentration range from 1 to $100 \mu\text{M}$ were between 1.8 and 6.9 % ($n=3$).

Three Vietnamese groundwater samples, that were done in standard system, also were tested for content of As(V). As(V) was detected and its concentration was quantified based on the calibration curve established in previous procedure. $0.17 \pm 0.02 \mu\text{M}$, $1.82 \pm 0.09 \mu\text{M}$ and $0.18 \pm 0.03 \mu\text{M}$ of As(V) was found in VN2, VN3 and VN5, respectively. The standard deviation was calculated for three subsequent analyses of the groundwater sample. Resulting electropherograms of the three ground water samples containing As(V) are shown in Figure 5.

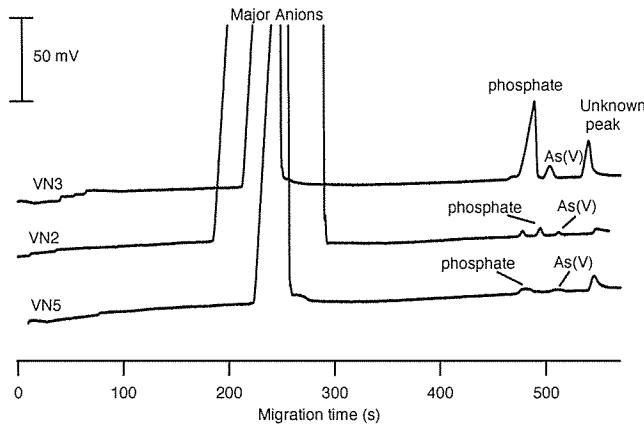


Figure 5. Determination of As(V) in 3 ground water samples from different places in Vietnam using portable capillary electrophoresis instrument. Electrolyte solution: 45 mM acetic acid, pH 3.2. Capillary: fused-silica, 50 μ m i.d., $L_{\text{total}}=60\text{cm}$ ($L_{\text{eff}}=53\text{cm}$) Sample injection: hydrodynamic, 20 cm for 60 s. Other conditions as for Figure 2.

As shown in Fig 5, the results were almost achieved compared to standard system. The system is absolutely proposed for portable arsenic screening analysis.

Conclusions

The study presented is to investigate the determination of trace levels of inorganic analytes in presence of high levels of major ions using capillary electrophoresis with contactless conductivity detection. The system for the portable analysis has been optimised by using 12V power supply battery. The determination of the inorganic anionic arsenic species of As(III) and As(V) was found possible in natural water samples with this simple and inexpensive method by using individually optimized methods. These have potential in environmental applications and may be used for fast screening of groundwater contaminated with arsenic. Notably, the obtained limited detection meets the proposed WHO requirements (50 μ g/L) but does not decrease to 10 μ g/L as guided by other countries including EPA-USA.

Acknowledgements

The authors would like to thank the Swiss Federal Commission for Scholarships for Foreign Students (ESKAS) to enable the postgraduate study for Nguyen Thi Anh Huong (Ref. No.: 2005.0339/Vietnam/OP), the Swiss National Science Foundation (Grant No.200020-105176/1 for partial financial support as well as Michael Berg (Swiss Federal Institute of Aquatic Science and Technology (EAWAG) - Switzerland), and Pham Thi Kim Trang and other researchers of Arsenic group from Research Centre for Environmental Technology and Sustainable Development (CETASD) – Hanoi.

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