

THE USE OF L-ASCORBIC ACID IN SPECIATION OF ARSENIC COMPOUNDS IN DRINKING WATER

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Arsenic speciation, besides total arsenic content determination, is very important in analysis of water, foodstuffs, and environmental samples, because of varying degrees of toxicity of different species. For such purpose hydride generation atomic absorption spectrometry can be used based on the generation of certain types of hydride, depending on the pH value and pretreatment in different reaction media. In this study, we have investigated the effect of L-ascorbic acid as the reaction medium as well as the pre-reducing agent in speciation of arsenic by hydride generation-atomic absorption spectrometry in order to determine monomethyl arsonic acid (MMA) in the presence of inorganic forms of arsenic.

KEY WORDS: Total arsenic, speciation, L-ascorbic acid, atomic absorption spectrometry, hydride generation

INTRODUCTION

Although content of arsenic in lithosphere (without atmosphere and hydrosphere) is relatively low ($5 \cdot 10^{-4}$ mass %) (1), high toxicity of arsenic demands continuous determination of total arsenic as well as its different species in the environment. Reported concentrations of arsenic in soil are between 0.1 and 40 mg kg⁻¹ (2), while in non-contaminated soil average concentration is 5- 6 mg kg⁻¹ (3,4). By using arsenic based pesticides, fertilizers, irrigation, fossil fuel burning, disposal of industrial and other waste products, humans are exposed to the action of arsenic in the environment (5). Arsenic concentration in the parts of plants used for human nutrition is usually low, even when grown on contaminated ground (6), despite the fact that higher concentrations are found in plants grown on contaminated soil near mines and smelteries (7-10). Monomethyl arsonic acid (MMA) and dimethyl arsinic acid (DMA) are better absorbed and also better assimilated (incorporated) by plants than arsenic inorganic compounds (11). Arsenic concentration in marine water systems is in the range of 2 - 3 µg/l (12), while in marine organisms it is in the range of 1-30 mg kg⁻¹ of arsenic (13) as arsenobetaine (14). Rivers, lakes and ground-

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water contain up to 100 $\mu\text{g/l}$ of arsenic, drinking water of Bangladesh and West Bengal, India contains more than 100 $\mu\text{g/l}$ of arsenic (15).

Arsenic is one of the elements of public concern because of its highly toxic and carcinogenic properties (I group) (16-19). Exposure to arsenic can cause a variety of adverse health effects, including dermal, respiratory, cardiovascular and gastrointestinal changes. Also, arsenic is genotoxic and mutagenic (20, 21). Inorganic forms are highly toxic, and arsenite is apparently more toxic than arsenate. The methylated organic species (MMA) and (DMA) are less toxic than the inorganic forms (20, 22, 23).

The Serbian Regulation on Hygienic Propriety of Drinking Water (25), European Union regulation (Directive EU 98/83/EC), directive of WHO (World Health Organization) and EPA (United States Environmental Protection Agency) prescribe maximum contaminant level of arsenic in drinking water of 10 $\mu\text{g/l}$. However, these standards define only total arsenic MRL (Maximum Residue Level), regardless of arsenic forms and without request of arsenic speciation. Besides, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry in toxicological profile for arsenic, specifies the level of 0.3 $\mu\text{g/kg}$ of inorganic form per day, as a limit under which there are no toxic effects in case of human oral consumption longer than one year (24).

Arsenic speciation can be very important in the analysis of water, foodstuffs, and environmental samples, besides of total arsenic analysis. Possible method used in the speciation of arsenic compounds is atomic absorption spectrometry with hydride generation, and it is based on the generation of certain types of hydride, depending on the pH value and pretreatment in different reaction media. The aim of this work was to explore the impact and the possible use of L-ascorbic acid as the reaction medium and the pre-reducing agent for hydride generation of different arsenic compounds in the process of speciation, in order to determine MMA in the presence of inorganic forms of arsenic.

Until now L-ascorbic acid was used as a chemical agent for arsenic preservation and stabilization in samples with arsenic concentrations 1mg/ml (30, 45, 46). L-ascorbic acid is also used as pre-reducing agent for the determination of total arsenic and total inorganic forms [As(III) and As(V)], in the presence of thiourea (28, 31) or in combination with KI (33, 34, 36, 38). In other cases, mixture of L-ascorbic acid and KI was used for reduction of inorganic As(V) and organic compound arsenic MMA by using cryogenic or HPLC separation (26, 27, 29, 32, 37). Anderson et al. (44) reported KI utilization in the determinations of total inorganic arsenic forms. In this paper, results of the study on the effect of L-ascorbic acid as the reaction medium and the pre-reducing agent are given. To the best of our knowledge, there are no data about the L-ascorbic acid usage in the determination of MMA in the presence of inorganic forms of arsenic by hydride generation-atomic absorption spectrometry.

EXPERIMENTAL

All chemicals were of analytical grade unless otherwise specified. Water used was obtained from a purification system (Elga) and the conductivity of reagent water was 0.05 $\mu\text{S cm}^{-1}$. The arsenic stock solutions were prepared as follows: 1 mg/ml As(V) ($\text{As}_2\text{O}_5 \cdot 2\text{H}_2\text{O}$ in H_2O) (Carlo Erba Analytical), 6.6495 mg/ml NaAsO_2 (Merck), 1mg/ml MMA and 0.039376g original solid disodium methyl arsonate hexahydrate >99% (Chem

Service) were dissolved in 10 ml reagent water; 1mg/ml DMA and 0.028568g original solid sodium cacodylat trihydrat >98% (Fluka) were dissolved in 10 ml reagent water. Working standard solutions were prepared daily by diluting appropriate volumes of stock solution in reagent water. Sodium hydroxide p.a. (Zorka Pharma), sodium tetrahydroborate p.a. (Fluka), hydrochloric acid 37% (Merck), potassium iodide p.a. (Fluka), L-ascorbic acid p.a. (Zorka Pharma). Reagent water without detected presence of arsenic compounds and drinking water with 3 and 4 $\mu\text{g/l}$ of total arsenic were used as samples.

Analyses were performed on an Atomic Absorption Spectrometer Shimadzu AA-680 with Hydride Vapour Generation (HVG-1 3 channels, Shimadzu) connected to the instrument. The sample solutions were pumped into a manifold where they reacted with acid and sodium tetrahydroborate solution. Using nitrogen, the generated arsines were swept to a gas-liquid separator and then to a heated T-shaped absorption cell.

The possibility of using L-ascorbic acid in speciation of As(V) and MMA in drinking water was investigated on three channels system with peristaltic pump (HVG-1) for hydride generation. Through the channel for the sample aspiration, water solutions of arsenic compounds or solutions of arsenic compounds with hydrochloric acid, L-ascorbic acid or KI were introduced. Hydrochloric acid was introduced through the second channel and through the third channel reduction solution of sodium tetrahydroborate. After appropriate investigation and statistical treatment ("t"-test), calibration curve was made using standard solution of As(III). During the determination of MMA it was necessary to wait longer for the hydride generation and extended washing of hydride system was also necessary.

RESULTS AND DISCUSSION

During investigation of the influence of L-ascorbic acid on hydride generation, through channel for acid were introduced different concentrations of L-ascorbic acid. The effect of 1-10% L-ascorbic acid on the absorbance signals, in the absence or presence of HCl in sample is shown in Fig. 1.

The absorption signals of As(III) and DMA increased with the increase of L-ascorbic acid concentration, while the response for As(V) and MMA was low, caused by the different degrees of protonation of arsenic compounds. Protonation and the formation of arsines are pH dependent. The pK values of arsenite-As(III), arsenate-As(V), MMA and DMA, are 9.23, 2.25, 2.60, 6.19, respectively (47). Under these conditions reduction products (arsines) appear, AsH_3 from arsenite-As(III) and arsenate-As(V) (boiling point - 55°C), CH_3AsH_2 from MMA (boiling point 2°C) and $(\text{CH}_3)_2\text{AsH}$ from DMA (boiling point 35.6°C) (47). All obtained signals were lower than in case when hydrochloric acid was used in hydride generation as reported previously (27). The obtained results were similar to those obtained by applying the other organic acids like oxalic acid, acetic acid, tartaric and citric acid. They were also in accordance with the investigation of Anderson et al. (44). In the case when HCl was present in the sample, higher values of analytical signals (Fig. 1, dashed line) were obtained and maximum values of relative sensitivity were obtained when lower concentrations of L-ascorbic acid was used (Fig. 1).

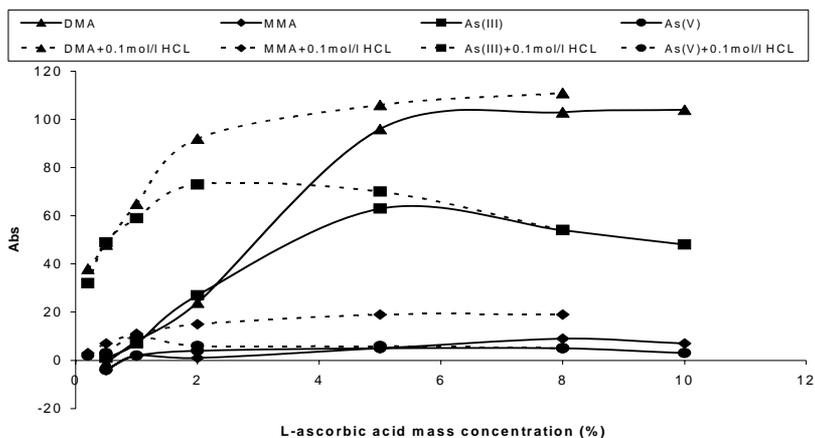


Fig. 1. Effect of L-ascorbic acid concentration on the absorption signals of As(III), As(V), MMA and DMA. Three-channel system: the first channel with sample of arsenic (10 $\mu\text{g/l}$ As each) in water (dashed line, sample in 0.1 mol/l HCl), the second channel with different concentrations of L-ascorbic acid and the third channel with 0.4% NaBH_4 in 0.5% NaOH

Similar effect was observed in the case when KI was added as pre-reducing agent in the samples (Fig. 2). There was no reduction of arsenic(V) compounds and MMA, because pH value was not sufficiently low. MMA was reduced partially when KI and 0.1 mol/dm³ HCl were added to the sample (Fig. 2, dashed line). Because of the relatively high pH value, the reduction of As(V) practically was not observed.

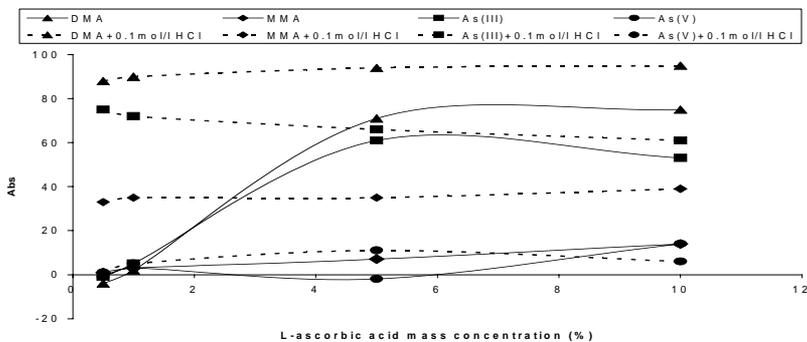


Fig. 2. Effect of L-ascorbic acid concentration on the absorption signals of As(III), As(V), MMA and DMA. Three-channel system: the first channel with sample of arsenic (10 $\mu\text{g/l}$ As each) + 1.5ml 40% KI in water (dashed line, sample in 0.1 mol/l HCl), the second channel with different concentrations of L-ascorbic acid and the third channel with 0.4% NaBH_4 in 0.5% NaOH

The effect of potassium iodide concentration on the degree of As(III), As(V), DMA and MMA reduction, without or with L-ascorbic acid (Fig. 3, dashed line), was studied. KI concentration varied within the range of 0.5-3% and results of this experiment are shown in Fig. 3. As(III) is the reduction form of arsenic and the presence of KI or L-ascorbic acid has no effect on reduction and generation of its hydride. In the presence of L-ascorbic acid, DMA was not reduced, neither hydride was generated, because the pH value of the solution was too low. In the absence of L-ascorbic acid in the sample, reduction of MMA was low or there was no reduction, while when L-ascorbic acid was added, reduction of MMA was complete. Reduction of As(V) was complete in the presence of L-ascorbic acid. Similar results were obtained by Anderson et al. for As(III) and As(V) in the absence of L-ascorbic acid (44). These characteristics of As(V) and MMA were used for the arsenic speciation, in order to determine MMA in the presence of inorganic forms of arsenic. The results of this investigation has shown that very good arsenic reduction was obtained when the KI concentration was about 1.5 %. Burguera et al. in their investigation (47) reported similar observations.

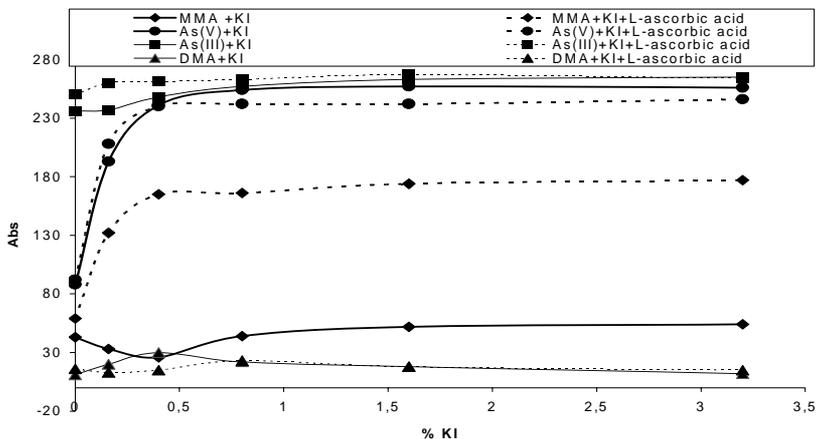


Fig. 3. Effect of KI mass concentration on the absorption signals of As(III), As(V), DMA and MMA. Three-channel system: the first channel with sample arsenic (20 $\mu\text{g/l}$ As each) + different concentration KI in 2 mol/l HCl (dashed line, sample with L-ascorbic acid), the second channel with 5 mol/l HCl and the third channel with 0.4% NaBH_4 in 0.5% NaOH

The influence of the time needed for complete reduction of As (V) and MMA under optimized experimental conditions is shown in Fig. 4.

Fig. 4 shows that at least 10 minutes were needed for the complete reduction of MMA and about 40 minutes for arsenate-As(V). DMA was not reduced, neither hydride was generated, because of the solution pH value (42, 44).

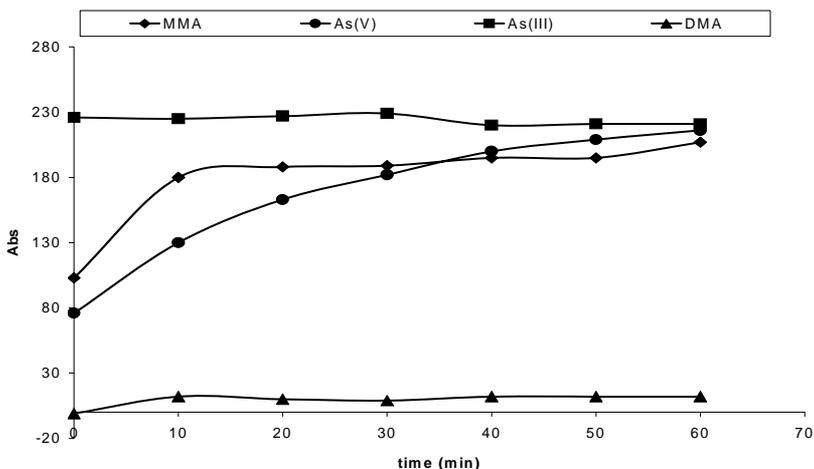


Fig. 4. Effect of time needed for reduction with KI on the absorption signals of As(III), As(V), MMA and DMA. Three-channel system: the first channel with sample arsenic (20 $\mu\text{g/l}$ As each) + 1ml 40% KI + 1ml 5% L-ascorbic acid in 2 mol/l HCl, the second channel with 5 mol/l HCl and the third channel with 0.4% NaBH₄ in 0.5% NaOH

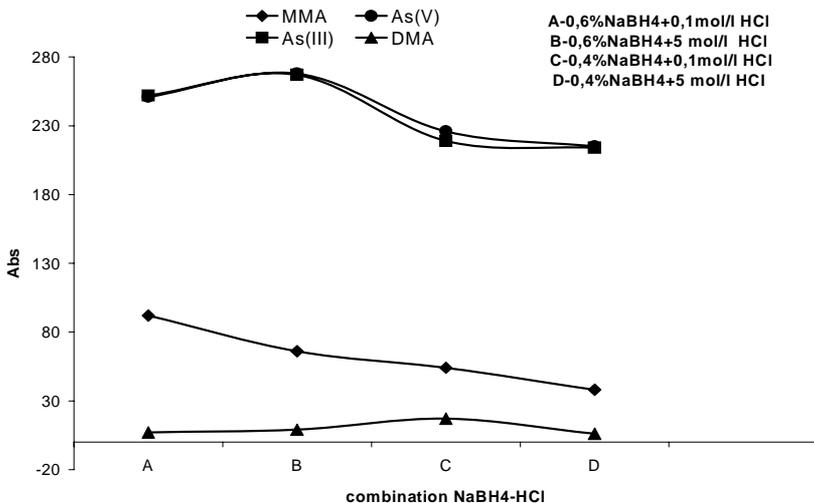


Fig. 5. Effect of different reaction media on the generation of arsines of As(III), As(V), MMA and DMA. Three-channel system: the first channel with sample of arsenic (20 $\mu\text{g/l}$ As each) + 0.125ml 40% KI in 5 mol/l HCl, the second and third channels are described as A, B, C and D

In an attempt to find optimal experimental conditions under which L-ascorbic acid was used in process of speciation of As(V) and MMA, four experimental conditions were applied (A, B, C and D) as shown in Fig. 5. Under condition of “D” (Fig. 5), MMA signal was low. The condition “D” was used for the determination of total inorganic arsenic [As(III) and As(V)], despite of the presence of L-ascorbic acid. In the case when L-ascorbic acid was added, MMA was determined too. Concentration of MMA was obtained as the difference between As(III)+As(V)+MMA in the presence of KI and L-ascorbic acid and As(III)+As(V) in the presence of KI only. Namely, the aim was to obtain minimal value of the analytical signal of MMA, not the maximal difference between the analytical signals of MMA and of inorganic arsenic compounds.

Using appropriate conditions developed in this study, two different samples were analyzed (reagent and drinking water). Under investigated conditions As(III) was completely reduced and its hydride was generated, while hydride was not generated in case of DMA (because of low pH) and only the content of As(V) and MMA were determined. As(III) and DMA behave equally both in the presence and in the absence of L-ascorbic acid, so investigation under chosen conditions had no effect. The results are shown in Table 1. After separate examination of all standard solutions (As(III), As(V) and MMA) for calibration curves construction and after the conduction of “t”-test ($t_{tab}=2.10$, degree of freedom was 18, number of replicate was $n=10$, $\alpha=0.05$), for calibration range of 0.004-0.018 mg/l t value of 0.21-2.07 was obtained. Values of “t”-test indicate that there is no significant difference between the previously mentioned standard solutions, so As(III) was chosen for the calibration curves definition.

Table 1. Results for water samples, % recovery and % RSD

Sample type ^a	Analyzed species ^b	Total arsenic concentration (ppb) before spiking	Added (ppb)		Total arsenic concentration (ppb) after spiking	Recovery ^c (%) (n=5)	RSD (%) (n=5)
			As(V)	MMA			
RW	As(V)	0.0	8.0	8.0	8.1	101.3	1.2
DW	As(V)	3.0	8.0	8.0	10.5	88.8	2.4
RW	As(V)	0.0	4.0	4.0	3.7	92.5	2.0
RW	As(V)+MMA	0.0	8.0	8.0	14.0	87.6	1.3
DW	As(V)+MMA	3.0	8.0	8.0	17.7	89.1	1.3
RW	As(V)+MMA	0.0	4.0	4.0	7.3	90.7	2.1
DW	As(V)+MMA	4.0	4.0	4.0	11.7	92.1	2.7

a- RW-reagent water and DW- drinking water,

b- reaction media is D (Fig. 5); for determination As(V) only KI is added, for determination As(V)+MMA KI+L-ascorbic acid are added,

c-recovery of analyzed species

Results reported in this study showed that the recovery of added spike was 82-101% and RSD (%) was 3.6, which are acceptable results for this level of content (ppb). It was confirmed that the addition of L-ascorbic acid to the samples as the pre-reducing agent enables the arsine generation from MMA and it was shown that these analyses can be used in speciation of arsenic compounds. Ascorbic acid is frequently applied as a pre-reductant for As(V) and MMA together with iodide in order to prevent the liberation of

iodine. The influence of ascorbic acid on the generation of hydrides of various arsenic species has not been previously studied in detail. In reference (35) it was mentioned that there is a possibility that this phenomenon is the result of better protonation in the presence of L-ascorbic acid or easier approach of the pre-reducing agent (KI) to methylated arsenic compounds. It is evident that in the process of reduction agent iodine was made and it was reduced with L-ascorbic acid into iodide, which entered into the process of reduction of arsenic compounds again. The detailed conclusions about the L-ascorbic acid influence could be obtained by the investigations of kinetics of these chemical reactions.

Based on the results obtained by the determination of As(V) and MMA in water samples it can be concluded that L-ascorbic acid can be used as the reaction medium and the pre-reducing agent for hydride generation of different arsenic compounds in the process of speciation, in order to determine MMA in the presence of inorganic forms of arsenic.

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ПРИМЕНА Л-АСКОРБИНСКЕ КИСЕЛИНЕ ПРИ ОДРЕЂИВАЊУ РАЗЛИЧИТИХ ОБЛИКА АРСЕНА У ВОДИ ЗА ПИЋЕ

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Приликом анализе различитих врста узорака вода, животних намирница и узорака из животне средине, указује се потреба да се осим одређивања садржаја укупног арсена изведе и одређивање различитих облика арсена. Атомска апсорпциона спектрометрија са грађењем хидрида је метода којом је могуће одредити различите облике арсена применом различитих услова стварања хидрида. Л-аскорбинска киселина се може употребити као реакциони медијум, односно као прередуковани агенс у стварању хидрида арсена током специјације. Циљ овог рада је да се испита могућност коришћења Л-аскорбинске киселине при одређивању органског облика арсена, монометил арсонске киселине (ММА), у води за пиће у присуству неорганског арсена (As(III) и As(V)).

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