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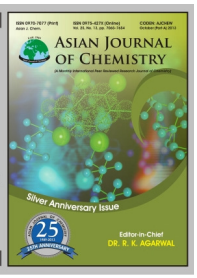

Arsenic Speciation in Drinking Tea Samples by Hydride Generation Atomic Fluorescence Spectrometry

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A simple, low cost and fast speciation method has been developed for the arsenic speciation by hydride generation atomic fluorescence spectrometry (HG-AFS). The contents of arsenic(III) and arsenic(V) was calculated by solving the simultaneous equations obtained at two different experimental conditions, *i.e.*, in the citric acid medium and thiourea-ascorbic acid medium. The total arsenic content was detected after the sample was digested with HNO_3 and H_2O_2 and prepared in thiourea-ascorbic acid medium. The content of the organic arsenic was calculated by subtracting the amount of inorganic arsenic from the total amount of arsenic. The detection limits of this method were 7.0×10^{-2} μg L^{-1} for As (III) and 9.5×10^{-2} μg L^{-1} for total inorganic arsenic, respectively. This method was applied to the arsenic speciation in six types of Chinese drinking tea without chromatographic and electrophoretic separation.

Key Words: Arsenic speciation, Drinking tea, Hydride generation atomic fluorescence spectrometry.

INTRODUCTION

Tea is drunk by about half of the world's population which is both widely cultivated[1] and consumed in many countries such as China, India, Japan, *etc*. Tea leaves contain high contents of amino acids, proteins, tea polyphenols and numerous essential elements. On the other hand, tea leaves accumulate heavy metal elements such as As, Hg, Pb and Cd, which may pose serious risk to the consumers' health. Therefore, intensive research has been carried out to investigate the contents of these heavy metals in tea leaves or drinking tea[2-8].

Arsenic has been assigned as a carcinogen to human by the International Agency for Research on Cancer (IARC). Toxicity research shows that the toxicity of arsenic is correlated with its chemical forms. The most toxic form of arsenic is the inorganic arsenic, *i.e*., As(III) and As(V), while the organic arsenic exhibits less toxicity. Therefore, arsenic speciation is more useful and scientific than the determination of total arsenic in evaluating the toxicity of samples. Recently, much effort has been devoted to developing methodologies for arsenic speciation in various samples especially in the edible foods[9-15]. To detect different chemical forms of arsenic, highly efficient separation techniques such as high performance liquid chromatography (HPLC)[16-18], ion chromatography (IC)[19,20], gas chromatography (GC)[21,22] and capillary electrophoresis (CE)[23] are usually coupled to the sensitive detection techniques including atomic fluorescence spectrometry (AFS)[12,16], atomic absorption spectrometry (AAS)[20,24], atomic emission spectrometry (AES)[21] and inductively coupled plasma mass spectrometry (ICP-MS)[19,22]. However, these methods reported previously are time-consuming, labour-intensive and costly since the chromatographic or electrophoretic separations are employed. Recently, a new hybrid technique, *i.e*., chip-based capillary electrophoresis (CBCE) coupling with AFS or MIP-OES (microwave induced plasma optical emission spectrometry) was described to achieve rapid speciation analysis of inorganic arsenic[25,26]. However, the chip-based methods are limited by the tedious chip fabrication and complicated design of chip-detector interface.

The main purpose of this work is to present a simple, low cost and rapid method suitable for the arsenic speciation in drinking tea. The methodology selected to do this present work is based on the different behaviour of arsenic species in a sensitive detection system like HG-AFS and thus two proportional equations that the fluorescence intensity as function of arsenic(III) and arsenic(V) concentration could be obtained for the same sample at two different experimental conditions. By solving the two simultaneous equations, the content of arsenic(III) and arsenic(V) in sample could be calculated. Additionally, the total content, *i.e*., the inorganic arsenic plus the organic arsenic could be detected after the sample was digested with HNO_3 and H_2O_2. Thus, the content of organic arsenic could be calculated by subtracting the inorganic arsenic content from the total content of arsenic.

EXPERIMENTAL

All chemicals used were of analytical grade unless mentioned otherwise. Doubly deionized water was used throughout. A stock standard As(III) solution (1.0 mg mL^{-1}) was prepared by dissolving 0.1325 g As_2O_3 in 10 mL of 10 % NaOH solution, neutralized with dilute HCl solution and diluted to 100 mL with water. A stock standard As(V) solution (1.0 mg mL^{-1}) was prepared by dissolving 0.2773 g Na_3AsO_4 with water and diluted to 100 mL. A 5.0 % (m/v) KBH_4 solution was prepared by dissolving 25.0 g KBH_4 and diluted to 500 mL with 0.5 % (m/v) NaOH solution. 1 % (m/v) thiourea-1 % (m/v) ascorbic acid and 0.1 mol L^{-1} citric acid was prepared in 5 % HCl.

An AFS-2202E Model atomic fluorescence spectrometry (Beijing Haiguang Instrumental Company, Beijing, China) equipped with an arsenic hollow cathode lamp (Beijing Haiguang Instrumental Company, Beijing, China) was used for the measurements of fluorescence intensity. Additionally, an automated intermittent flowing system and two gas-liquid separators were integrated with AFS for hydride generation and gas-liquid separation. Argon gas was used as carrier gas and shield gas for sweeping the arsenic hydride to the atomizer.

Procedure: Using the manifold shown in Fig. 1, the samples, carrier solution and KBH_4 were introduced into the HG-AFS system and the fluorescence intensity was measured. An automated intermittent flowing system was used throughout according to the programs shown in Table-1. Specifically, the sample and the reductant (KBH_4) were pumped and merged at a flow rate of 0.14 mL s^{-1} for 10 s, then the sampling tube was transferred to the carrier solution (5 % hydrochloric acid) from the sample such as to introduce carrier solution and KBH_4 at a flow rate of 0.14 mL s^{-1} for 16 s. Thus, sample was mixed with KBH_4 to generate arsenic hydride and hydrogen gas. Using the argon gas as carrier gas, the generated arsenic hydride and hydrogen gas were then subjected to a two-step gas-liquid separation in sequence to ensure efficient elimination of moisture in the gas mixture. The hydrogen gas and arsenic hydride were swept to the atomizer by the carrier gas and the atomized arsenic was excited by the radiation emitted from the arsenic hollow cathode lamp. The emitted fluorescence was recorded and processed by the software that controls the operation of the AFS-2202E system. The instrumental conditions were shown in Table-2.

TABLE-1
WORKING PROGRAMS OF THE INTERMITTENT FLOWING SYSTEM

Step	Reagents	Flow rate (mL s^{-1})	Time (s)	Function
1	Sample KBH_4	0	6	Inserting tube into sample and reductant solution
2	Sample KBH_4	0.14	10	Introduction of sample and reductant
3	Carrier KBH_4	0	6	Transferring tube into carrier solution from sample
4	Carrier KBH_4	0.14	16	Introduction of carrier and reductant; return to step 1

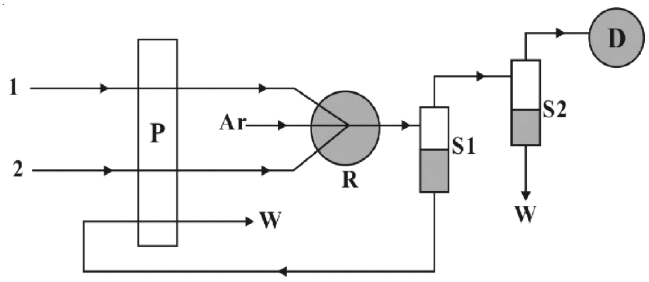

Fig. 1. Schematic diagram of HG-AFS for the arsenic speciation. P, peristaltic pump; 1, sample or carrier solution; 2, reductant solution (KBH_4); Ar, argon gas; R, reactor; S1 and S2, gas-liquid separators; D, AFS detector; W, waste

TABLE-2
INSTRUMENTAL CONDITIONS EMPLOYED FOR THE DETERMINATION OF As BY HG-AFS

Instrumental conditions	Value
High voltage of PMT (V)	300
Height of atomizer (mm)	10
Lamp current (mA)	60
Delay time (s)	1.0
Read time (s)	10
Flow rate of carrier gas (mL min^{-1})	400
Flow rate of shield gas (mL min^{-1})	1000

Principle of arsenic speciation by HG-AFS: This method is based on the simultaneous proportional equations obtained at two different experimental conditions. In condition A, *i.e.*, when the sample was prepared in thiourea-ascorbic acid medium, As(V) in sample could be reduced to As(III). Therefore, the fluorescence intensity is proportional to the total arsenic concentration in condition A, *i.e.*,

$$F_{(A)} = K_1 [C_{As(III)} + C_{As(V)}] \quad (1)$$

where $F_{(A)}$ is fluorescence intensity in condition A, K_1 is a function of fluorescence quantum yield, the light intensity emitted from hollow cathode lamp, the absorption coefficient of arsenic and other experimental conditions, $C_{As(III)}$ and $C_{As(V)}$ are the concentration of As(III) and As(V), respectively.

In condition B, *i.e.*, when the sample was prepared in the citric acid medium, As(V) reacts with KBH_4 to generate arsenic hydride at a reaction velocity much lower than As(III). Therefore, the signal produced by As(III) is much higher than that produced by As(V) in citric acid medium provided that the concentration of these two species is equal and the fluorescence intensity should be proportional to the concentration of corresponding species, *i.e.*,

$$F_{As(III)(B)} = K_2 C_{As\,(III)} \text{ and } F_{As(V)(B)} = K_3 C_{As(V)} \quad (2)$$

where $F_{As(III)\,(B)}$ and $F_{As(V)(B)}$ are the fluorescence intensity produced by As(III) and As(V) in condition B, respectively. Therefore, in the citric acid medium, the fluorescence intensity emitted from a sample containing both As(III) and As(V) should be expressed as follows:

$$F_{(B)} = K_2 C_{As\,(III)} + K_3 C_{As\,(V)} \quad (3)$$

K_1, K_2 and K_3 can be determined with the help of graphs of F (fluorescence intensity) *versus* the concentration of corresponding arsenic species in the thiourea-ascorbic acid and the citric acid medium. Therefore, $C_{As(III)}$ and $C_{As(V)}$ in the same

sample could be determined by solving the simultaneous equations (eqns. 1 and 3).

All of the arsenic species in the sample, *i.e.*, As(III), As(V) and the organic arsenic could be reduced to As(III) by digesting the sample with HNO_3 and H_2O_2 followed by preparing it in thiourea-ascorbic acid meidum. Thus, the total arsenic could be determined in the thiourea-ascorbic acid medium after being digested by HNO_3 and H_2O_2. Therefore, the content of the organic arsenic in sample could be calculated by subtracting the amount of inorganic arsenic from the total amount of arsenic.

RESULTS AND DISCUSSION

Effect of KBH_4 concentration: In HG-AFS, KBH_4 is used not only as a reductant for generation of arsenic hydride, but also as the hydrogen source to sustain the argon-hydrogen flame. As a consequence, the concentration of KBH_4 may pose significant effect on fluorescence intensity. As shown in Fig. 2, the fluorescence intensity increased with the increase of KBH_4 concentration when the concentration of KBH_4 was less than 5 %. This may be due to the increased arsenic hydride generated at higher KBH_4 concentrations. However, the fluorescence intensity decreased with the increase of KBH_4 concentration when the concentration was higher than 5 %, this may be attributed to the diluted arsenic hydride caused by the increased hydrogen amount generated at higher KBH_4 concentrations. Therefore, 5 % KBH_4 was selected in this work.

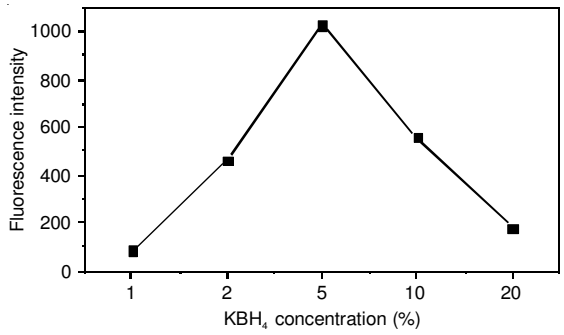

Fig. 2. Effect of KBH_4 concentration on fluorescence intensity. Carrier solution: 5 % hydrocloric acid, citric acid: 0.1 mol L^{-1}, As(III): 10 μg L^{-1}

Effect of citric acid concentration: The effect of citric acid concentration on fluorescence intensity was investigated with the citric acid concentration in the range of 0.05-0.5 mol L^{-1}. As shown in Fig. 3, the maximum fluorescence intensity was obtained with 0.1 mol L^{-1} citric acid. Hence, 0.1 mol L^{-1} citric acid was chosen in this work.

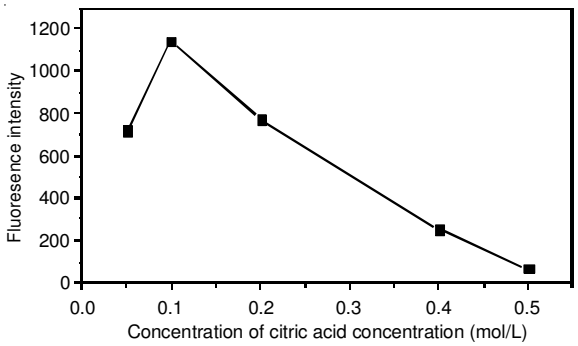

Fig. 3. Effect of citric acid concentration on fluorescence intensity. KBH_4: 5 %, other conditions are the same as in Fig. 2.

Effect of hydrochloric acid concentration: In this present work, hydrochloric acid was used as carrier solution as well as sample medium. The effect of hydrochloric acid concentration in the range of 0-15 % on the fluorescence intensity was investigated. As shown in Fig. 4, the fluorescence intensity increased rapidly with the increase of hydrochloric acid concentration when the hydrochloric acid concentration was in the range of 0-5 %. However, the fluorescence intensity decreased with the increase of hydrochloric acid concentration in the range of 5-15 %. The maximum fluorescence intensity was obtained with 5 % hydrochloric acid. Therefore, 5 % hydrochloric acid was selected as the carrier solution and sample medium in this work.

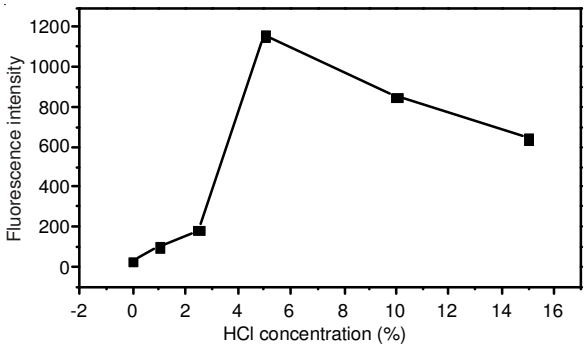

Fig. 4. Effect of HCl concentration on fluorescence intensity. KBH_4: 5 %, other conditions are the same as in Fig. 2

Effect of interference of foreign species: HG-AFS is characterized by the high selectivity. In this work, only those metal ions that may form hydride were prepared to study the effect of these foreign species on the determination of 10 μg L^{-1} As(III). Foreign specie was considered not to interfere with the determination of As(III) when the error caused by the foreign specie was within 5 %. Under the optimum conditions described above, 5.0×10^4 μg L^{-1} Cu^{2+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ag^{+}, Se^{4+} and Cr^{3+}, 1.0×10^4 μg L^{-1} Pb^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ge^{2+} and Cd^{2+} and 5.0×10^3 μg L^{-1} Sn^{4+} and Hg^{2+} didn't interfere with the determination of 10 μg L^{-1} As(III). These results indicate that the present method is selective in arsenic speciation.

Calibration

As(III) and As(V) solutions with different concentrations in the range of 0-10 μg L^{-1} were prepared and the fluorescence intensity in two different media was measured under the optimum conditions described above. The fluorescence intensity as a function of As(III) and As(V) concentration were obtained as the following:

$$F_{(A)} = 120.9[C_{As(III)} + C_{As(V)}] + 54.2 \quad r = 0.997 \quad (4)$$

$$F_{As(III)\,(B)} = 128.4\, C_{As(III)} + 30.5 \quad r = 0.997 \quad (5)$$

$$F_{As(V)\,(B)} = 4.6\, C_{As(V)} + 1.7 \quad r = 0.993 \quad (6)$$

A and B represent two different experimental conditions, *i.e.*, preparing the sample in 1 % thiourea-1 % ascorbic acid medium and 0.1 mol L^{-1} citric acid medium, respectively. The working equations thus could be derived as:

$$F_{(A)} = 120.9\,[C_{As(III)} + C_{As(V)}] + 54.2 \quad (4)$$

$$F_{(B)} = 128.4\, C_{As(III)} + 4.6\, C_{As(V)} + 32.2 \quad (7)$$

TABLE-3
RESULTS OF ARSENIC SPECIATION IN SIX TYPES OF DRINKING TEA

Sample (No.)	As(III)			As(V)			Organic As		
	Found (μg/L)	Added (μg/L)	Recovery (%)	Found (μg/L)	Added (μg/L)	Recovery (%)	Found (μg/L)	Added[a] (μg/L)	Recovery (%)
1	4.6	5	92.6	57.4	50	110.5	23.3	20	94.7
2	2.6	5	95.8	57.4	50	112.9	23.3	20	90.2
3	0.3	5	112.3	58.6	50	105.3	22.3	20	96.6
4	5.2	5	97.5	56.0	50	91.2	11.4	20	105.5
5	7.0	5	108.1	59.0	50	99.3	13.7	20	91.8
6	14.4	5	110.4	59.6	50	92.6	15.2	20	102.0

[a]Concentration of arsenic added in sample calculated from the added amount of sodium dimethylarsinate before the sample was digested by HNO_3 and H_2O_2.

The detection limits, based on the $3S^b$ criterion (S^b is the concentration corresponding to the standard deviation obtained from determining blank solution 11 times), were 7.0×10^{-2} μg L^{-1} for As(III) and 9.5×10^{-2} μg L^{-1} for total inorganic arsenic.

Application: This method was applied to the arsenic speciation in six types of drinking tea. A suitable amount of 2 g tea leaves was accurately weighed into a beaker and 80 mL of water at a temperature of 100 ± 0.5 ºC was added to extract arsenic for 20 min. After being cooled to room temperature in running cold water, the filtrate was then transferred into a 100 mL volumetric flask and diluted to 100 mL.

A suitable amount of above-mentioned solution was accurately transferred into a volumetric flask and prepared in 1 % thiourea-1 % ascorbic acid. $F_{(A)}$ was then measured according to the procedure described above. Another equal amount of solution was prepared in 0.1 mol L^{-1} citric acid and $F_{(B)}$ was measured. The contents of inorganic As(III) and As(V) in samples could be calculated from eqns. 4 and 7. The results were shown in Table-3.

To determine the organic arsenic in samples, a suitable amount of sample solution was accurately transferred into a 100 mL beaker and 5 mL of concentrated HNO_3 was added. After the solution was heated on a hot plate of about 100 ºC for 1 h, 1.0 mL of H_2O_2 was added and the solution was heated again to digest the sample until the solution was clear. The solution was then prepared in 1 % thiourea-1 % ascorbic acid and the total arsenic concentration was determined. The content of organic arsenic was calculated by subtracting the amount of total inorganic arsenic from the total arsenic concentration. The results were shown in Table-3.

Conclusion

We developed a method for arsenic speciation, with the advantages of simplicity, low cost and fast analysis speed over those methods coupling with gas chromatography, high performance liquid chromatography, capillary electrophoresis and chip-based electrophoresis. This method was used to determine As(III), As(V) and organic arsenic in drinking tea without chromatographic or electrophoretic separations. Furthermore, this method could also be applied to the arsenic speciation in environmental waters, foods, medicines and so on. Additionally, since this method was coupled with an automated intermittent flowing system for introducing the sample, carrier and reagents, micro-column filling with various materials could be integrated into the manifold for on line separation and concentration of arsenic species in complicated samples.

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