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Determination of inorganic arsenic species by hydride generation atomic absorption spectrophotometry and cathodic stripping voltammetry

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ABSTRACT: Flow injection-hydride generation-atomic absorption spectrophotometry (FI-HG-AAS) and square wave cathodic stripping voltammetry (SWCSV) were compared to detect inorganic arsenic species in lemongrass and turmeric. Two species, arsenite (As^{III}) and arsenate (As^{V}), were considered as they are known to occur in most terrestrial plants. As^{III} and total water-soluble inorganic arsenic (TAs_{inorg}) were determined under different conditions. As^{III} was selectively determined by using a soft generation condition, i.e., low HCl concentration, whereas TAs_{inorg} was determined after pre-reduction of As^{V} to As^{III} with a KI/ascorbic acid mixture. The As^{V} content was estimated as the difference between both measurements. Under optimal conditions, the limits of detection (LOD) by FI-HG-AAS were 0.02 and 0.03 μg/l for As^{III} and TAs_{inorg}, respectively. Relative standard deviations ($n = 9$) of less than 4% were obtained for both inorganic arsenic species. The accuracy was also verified by analysing spiked samples and certified reference material: CTA-VTL-2 (Virginia Tobacco leaves). The recoveries of both species were found to be between 90 and 115%. The determination of inorganic arsenic species by SWCSV in the samples is based on the formation of a copper-arsenic intermetallic compound at the hanging mercury drop electrode (HDME) during the preconcentration step. Only As^{III} was deposited on the Hg electrode when Cu was present in the HCl medium. TAs_{inorg} can be determined by reducing As^{V} to As^{III} with sodium thiosulphate. As^{V} is quantified as the difference. At optimum conditions, the LOD for As^{III} and As^{V} were 0.5 and 0.4 μg/l, respectively. Relative standard deviations ($n = 10$) of less than 5% were obtained and the method was validated by analysing the spiked samples and certified reference material. FI-HG-AAS showed better LOD than SWCSV for both inorganic arsenic species. There was, however, a strong agreement between TAs values obtained by using FI-HG-AAS and SWCSV technique in lemongrass, turmeric, and the certified reference material (CTA-VTL-2). As^{V} was the main inorganic arsenic species found in lemongrass and turmeric. The results confirm that the As content of both samples do not exceed the food safety limits for Thailand and several countries.

KEYWORDS: lemongrass, turmeric, food safety, heavy-metal toxicity, FI-HG-AAS, SWCSV

INTRODUCTION

Arsenic is a trace element which has generated increased interest in recent years due to its toxicity. It can enter terrestrial and aquatic environments through both natural formation and anthropogenic activity[1]. As toxicity depends not only on the total concentration but also on its chemical form[2]. Inorganic arsenic species are also known to be more toxic than organic ones. Arsenite (As^{III}) and arsenate (As^{V}) are the most toxic species and As^{III} is reported to be at least 60 times more toxic than As^{V} [3]. The methylated forms of arsenic monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), and trimethylarsine oxide (TMAO) are less toxic; followed by arsenocholine (AsC), arsenobetaine (AsB), and finally the arsenosugars, which are regarded as being non-toxic[4]. The inorganic compounds of arsenic (As^{III} and As^{V}) have been classified as carcinogenic whereas MMAA and DMAA have been identified as possible cancer promoters[5].

The determination of total concentration of the element arsenic was previously considered to be sufficient for critical and environmental considerations[1]. Since arsenic forms own great differences

related to their metabolism and toxicity, it is necessary to determine the concentration of individual species (arsenic speciation analysis) for a reliable assessment on the environmental impacts and health risks.

Several techniques have been developed to determine the concentrations of different arsenic compounds. The most widely used analytical methods for element speciation are hyphenated techniques, favoured for both the efficient separation and elemental-specific detection[6]. Most suitable method currently used for arsenic speciation are based on the combination of HPLC with very sensitive and element-specific detection methods such as high-performance liquid chromatography with a flame atomic absorption spectrophotometer (HPLC/AAS)[7], high performance liquid chromatography with atomic fluorescence spectroscopy (HPLC/AFS)[8], or high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC/ICP-MS)[9].

To improve sensitivity and eliminate matrix effects, hydride generation (HG) technique has been employed as a convenient link with the chromatographic separation[10]. Hydride generation technique is the most popular sample derivatization method used for arsenic detection since Holak's first report in 1969[11]. The developed method made use of $NaBH_4$ or KBH_4 to produce arsines to separate arsenic from the liquid products of the reaction. These arsines were subsequent detected by a variety of detectors to become on-line combinations such as HPLC/HG/AAS[12], HPLC/HG/AFS[13], or HPLC/HG/ICP-MS[9].

For samples containing only inorganic arsenic, i.e., As^{III} and As^{V}, chromatographic separation is not necessary. Hydride generation-atomic absorption spectrometry (HG-AAS) technique based on arsine generation under controlled pH conditions or different reaction media has therefore been developed to directly determine of both oxidation states of inorganic arsenic, of which the experiments were divided into two stages after extraction: (i) selective determination of As^{III} under controlled pH condition; and (ii) determination of total arsenic (TAs, i.e., the sum of As^{III} and As^{V}) after pre-reduction of As^{V} to As^{III} with any reducing agents. The As^{V} content was estimated by the difference of both measurements[10]. Alternatively, total arsenic can also be obtained by digestion with acids. Although HG-AAS technique is accepted to be able to accurately measure arsenic in environmental samples to parts per billion (ppb) concentration levels, several drawbacks have been reported: (i) it is limited to the materials that form volatile arsines; (ii) reaction conditions have to be strictly controlled; (iii) the presence of certain interfering elements can reduce the efficiency of HG; and (iv) the method is laborious. Moreover, the development of this method requires important investing and high running costs and limits their use in most laboratories[14,15].

Electrochemical techniques are considered to be an alternative way commonly used to differentiate inorganic arsenic species. These techniques are well known as a simple, sensitive, and inexpensive methods to speciate arsenic directly since As^{III} and As^{V} have different electroactivity[16,17]. Stripping voltammetric techniques are considered to be an attractive method for the determination of some arsenic species in environmental samples with the preconcentration of the analyte on the working electrode prior to the actual determination step during the potential scan. They have been applied to determine trace arsenic such as polarography, cyclic voltammetry, anodic stripping voltammetry (ASV), and cathodic stripping voltammetry (CSV). In ASV (anodic stripping voltammetry), As^{III} or As^{V} is initially reduced to As^{0} (accumulation step) at a sufficiently negative potential and a film is formed on the surface of the working electrode (generally gold). In the subsequent reoxidation (stripping step) by scanning to more positive potential, the anodic current of arsenic can be measured in the voltammogram[3]. In CSV (cathodic stripping voltammetry), the accumulation step involves the use of an adsorption or deposition inducer (usually Cu or Se) to immobilize the analyte on the working electrode (generally hanging mercury drop electrode, HMDE). The redissolution occurs with more negative potential scan[3]. Ferreira and Barros stated that in the determination of inorganic arsenic species using anodic stripping voltammetric mode, As^{III} can be reduced to the element (As^{0}) at potential 0.25 V in acidic solution, deposited onto a solid electrode such as gold, platinum, or copper and then stripped off using more negative potential. However, they found that frequent problems associated with As^{V} at solid electrodes such as memory effect limit the sensitivity and give poor precision, which makes this approach inconvenient for routine analysis[17]. In an attempt to avoid the problems, CSV mode at an HMDE has been used to determine arsenic, utilising the reaction between arsenic and copper or selenium to form an intermetallic compound (Cu_xAs_y) that can be preconcentrated on the HMDE and then cathodically stripped off. To reach a suitable speci-

ation analysis, two methods (FI-HG-AAS and CSV) commonly used for As speciation were selected here for a comparative study of their applicability and limitations for analysing lemongrass and turmeric grown on high risk arsenic-contaminated area in terms of speciation, total water-soluble inorganic arsenic (TAs_{inorg}), and total arsenic (TAs).

MATERIALS AND METHODS

FI-HG-AAS

An atomic absorption spectrophotometer (AAS), Perkin Elmer AAnalyst 800, equipped with an electrically heated quartz tube furnace with an arsenic electrodeless discharge lamp (As-EDL) as a radiation source. Arsine generation was performed with a Perkin Elmer Model FIAS-100 Flow-injection system equipped with a PTFE membrane gas-liquid separator. The device and instrumental parameters were as recommended by the manufacturer (Table 1). The solutions of acid and reductant were pumped at the rate of 10 and 6 ml/min, respectively.

CSV

A Potentiostat/Galvanostat AUTOLAB model PGSTAT 10 was used for all voltammetric measurements, interfaced with the multi-mode electrode stand model 663 VA (Metrohm) composed of an HMDE as working electrode, an Ag/AgCl/3 M KCl electrode as reference electrode, and a Pt wire as auxiliary electrode. The electrode cell was equipped with a nitrogen purge tube to remove oxygen prior to sample analysis as well as gaseous sulphur compounds produced during the reduction step in the total arsenic measurement. The volume of sample solution was 10 ml. Electrochemical parameters for cathodic stripping voltammetry are shown in Table 2. In addition, since the optimization revealed that a square wave provides better results, the CSV system for speciation will subsequently be referred to as square wave cathodic stripping voltammetry (SWCSV). All mentioned potentials are versus Ag/AgCl/3 M KCl as a reference electrode.

Table 1 Operating conditions of the FI-HG-AAS system.

Parameters	Setting
Spectrophotometer	
Wavelength	193.7 nm
Slit width	0.7 nm
EDL current	300 mA
Energy	35
Integration time	15 s
Read time	20 s
Signal measurement	Peak-area absorbance
Hydride generation	
Sample volume	500 µl
Quartz cell	15 cm path length × 8 mm i.d.
Heating	Electrothermal
Temperature	900 °C
Argon flow rate	40 ml/min
Reductant conc. ($NaBH_4$)	0.3% (w/v) in 0.05 M NaOH
Reductant flow rate	6 ml/min
HCl conc.	5% (v/v)
HCl flow rate	10 ml/min

Table 2 Operating conditions of the CSV system.

Parameters	As^{III}	TAs
Deposition potential (E_d)	−0.38 V	−0.39 V
Deposition time (t_d)	30 s	240 s
Equilibration time (t_{eq})	15 s	15 s
Frequency (f)	200 Hz	200 Hz
Step potential (E_s)	2 mV	2 mV
Amplitude (A)	40 mV	40 mV
Stirring	2000 rpm	2000 rpm
Drop size	0.52 mm^2	0.52 mm^2
Cu^{II} conc.	45 mg/l	600 mg/l
HCl conc.	2 M	2 M
$S_2O_3^{2-}$ conc.	–	3 mM

Reagents

All chemicals obtained from Merck, Sigma and Aldrich were of analytical reagent grade and were used without further purification. Standard solutions were prepared daily from 1000 mg/l stock solutions by dilution with distilled de-ionized water. A 1000 mg/l stock solution of As^{III} was made up by the dissolution of 1.3201 g of reagent grade As_2O_3 in 25 ml of 20% (w/v) NaOH solution, followed by neutralization with 20% (v/v) H_2SO_4 and diluting to 1000 ml with 1% (v/v) sulphuric acid. To prepare a 1000 mg/l stock solution of As^V, 1.5336 g of reagent grade As_2O_5 was dissolved in 1000 ml of de-ionized water.

$NaBH_4$ solutions were prepared daily by dissolving an appropriate amount of powdered $NaBH_4$ in 0.1% (w/v) NaOH. Solutions of HCl were prepared by adequate dilution of concentrated HCl (37% v/v) in de-ionized water. For SWCSV, the solution of 15 000 mg/l of Cu^{II} was prepared by dissolving the appropriate amount of $CuCl_2 \cdot 2\,H_2O$ in 250 ml of a 0.5% solution of HCl in de-ionized water. The certified reference material (VTL-2, Virginia Tobacco Leaves) was purchased from Poland (Warszawa, Poland).

Sample collection and preparation

Lemongrass and turmeric were sampled in high risk area with a random sampling procedure. Each sampling point was identified by using the global positioning system. The samples were collected by hands protected with plastic gloves and carefully packed into plastic bags after cleaning with de-ionized water and weighing. The samples were stored in a cold box at 4 °C and transported to the laboratory where samples were washed again with de-ionized water, then homogenized with a high speed homogenizer and frozen at −20 °C. After that they were freeze-dried at 22 °C with final pressure of 6.2×10^2 mbar. The water was removed by freeze-drying because the water removed by an oven is not recommended for arsenic speciation analysis as heat may lead to the transformation of arsenic species. The dried samples were finally ground to powder with mortar and pestle. Water content values were found to be within the 84–90% range for lemongrass and 83–91% for turmeric.

Sample extraction for arsenic speciation

A portion of the freeze-dried sample (about 100 mg) was weighed into a centrifuge tube and nano-pure water (5 ml) was added. The extraction was performed by placing the tubes in an ultrasonic bath for 1.5 h. The mixture was centrifuged (50 000g, 30 min), and the supernatant decanted from the pellet which was further extracted for two more times. The three supernatants were combined to obtain the total volume of 10 ml in volumetric flask just before arsenic speciation analysis (As^{III} and As^{V}) by FI-HG-AAS and SWCSV, respectively[18]. Water is an extraction solvent for arsenic in plant samples under study, with the found recovery of not less than 94%.

Sample digestion for TAs analysis

A subsample of 0.25 g (dry weight) was transferred into 50 ml beakers and then 5 ml HNO_3, 1 ml $HClO_4$, and 0.5 ml H_2SO_4 were added. The mixture was heated at 100 °C to remove the acid and concentrated to about 1 ml. The clear solution was diluted to 25 ml with deionized water before analysis. The total arsenic content was then measured by FI-HG-AAS and SWCSV, respectively[19].

Speciation data manipulation

The inorganic arsenic species in lemongrass and turmeric were compared between the two methods. Speciation of inorganic arsenic (As^{III}, As^{V}) was carried out in two stages, As^{III} and total inorganic As (TAs_{inorg}). As^{V} was determined by subtraction ($As^{V} = TAs_{inorg} - As^{III}$).

As speciation by FI-HG-AAS

For the determination of As^{III} by HG-AAS, an aliquot (1 ml) of the extracted samples was transferred into a 10 ml PTFE tube and then diluted to 10 ml with 10% v/v HCl (recommended sample diluent). In cases of total water-soluble inorganic arsenic (TAs_{inorg}) and total As (TAs), an aliquot (1 ml) of the extracted samples was transferred into a 10 ml PTFE tube and then 1 ml concentrated HCl followed by 1 ml of a solution containing 3% w/v KI/ascorbic acid were added. After 45 min at ambient temperature, it was diluted to 10 ml with 10% v/v HCl.

As speciation by CSV

For the determination of As^{III} by SWCSV, an appropriate amount of standard or extracted sample was transferred to the analysis vessel containing deionized water to reach the total volume of 10 ml, followed by the addition of concentrated HCl to a concentration of 2 M HCl supporting electrolyte and 1000 mg/l Cu^{II} solution to obtain the final concentration of 45 mg/l. Sample was purged for 30 s with N_2 before SWCSV analysis. The TAs_{inorg} and TAs were determined in the same way as As^{III}, except that 0.5 M thiosulphate as reducing agent was added after HCl to obtain the concentration of 3 mM, followed by Cu^{II} to reach the concentration of 600 mg/l.

Optimization of FI-HG-AAS parameters

The chemical and physical parameters were experimented to achieve the optimum conditions for the analytical performance of the FI-HG-AAS system for the reliable quantification of as in plant samples. To obtain a stable and robust analytical signal, the quartz cell atomizer and the EDL-lamp were allowed to warm up for at least 45 min before starting a measurement sequence. Additionally, the response of the FI-HG-AAS system to a 10 μg/l As^{III} standard solution was determined each day before starting measurements. Although the relative standard deviations (%RSD) of 10 μg/l As^{III} standard solution were comparably low (< 2%), peak height provides about 4 times lower sensitivity than that from peak area. Hence peak area was used throughout all experiments for quantifying instrumental response. All instrumental parameters were optimized with 10 μg/l As^{III} and As^{V} standard solutions.

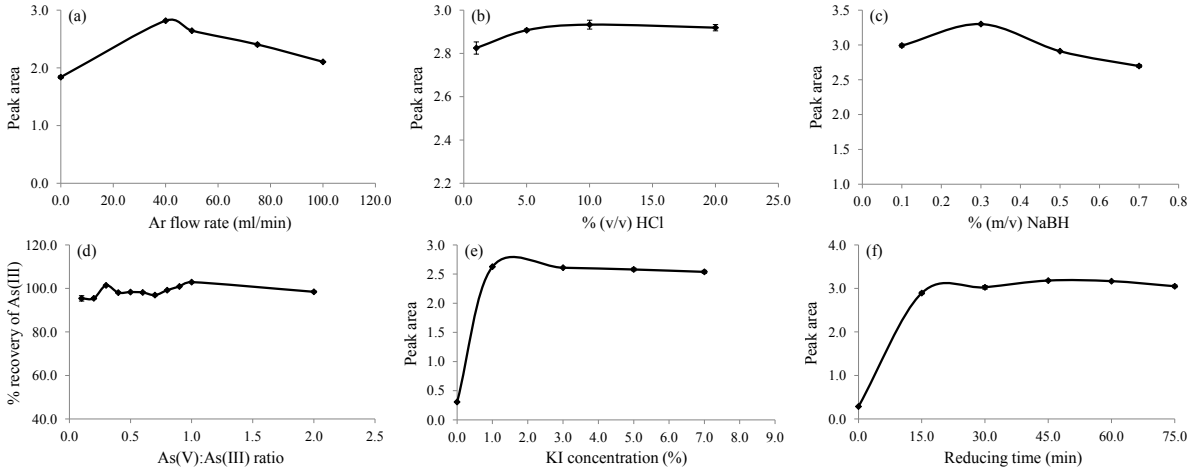

Fig. 1 The influence of (a) argon gas flow rate, (b) HCl concentration, and (c) $NaBH_4$ concentration on signal intensities of 10 μg/l As^{III} standard solution, and (d) effect of As^{V}: As^{III} ratio, (e) KI/ascorbic acid concentration, and (f) reducing time.

RESULTS AND DISCUSSION

Gas flow rate

Argon is needed as a carrier gas to transport the formed hydride to the quartz cell atomizer. The carrier gas flow rate generally influences the sensitivity of the analysis, since it affects resting time of arsenic atoms in the atomizer cell. The gas flow rate settings in the instrument can only be increased with 40 ml/min increments up to a maximum value of 200 ml/min. As shown in Fig. 1a, when the gas flow rate was increased from 40–100 ml/min, the signal intensities decreased significantly. A gas flow rate of 40 ml/min was therefore used for all further investigations.

HCl concentration

The HCl concentration is an important parameter because it significantly influences the HG efficiency. The concentration of HCl, as a carrier solution, was investigated within the range of 1–20% (v/v) to obtain the highest signal intensity. As shown in Fig. 1b, the signal intensity for As^{III} standard solutions reached a plateau at the HCl concentration of 10% (v/v), the HCl concentration of 5% (v/v) was chosen for all further investigations since the signal intensity was not significantly different from that in HCl concentration of 10% (v/v).

$NaBH_4$ concentration

The $NaBH_4$ concentration is also important in influencing the HG efficiency. $NaBH_4$ concentrations between 0.1 and 0.7% (w/v) were investigated using the optimized HCl concentration of 5% (v/v). As shown in Fig. 1c, the signal intensities increased up to a concentration of 0.3% (w/v) $NaBH_4$ and then significantly decreased. Hence $NaBH_4$ of 0.3% (w/v) was used as an optimal concentration for further investigations.

Effect of As^{V}:As^{III} ratio

For the selective determination of As^{III}, the interference effect from As^{V} was investigated. The evaluations were made from different mixtures of 10 μg/l As^{III} with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 20 μg/l of As^{V}. Fig. 1d shows that As^{V} does not interfere on As^{III} detection even at doubled concentration. The proposed method for As^{III} speciation can therefore be applied to samples with relatively high As^{V} levels.

Effect of KI:ascorbic acid concentration

Although the direct determination of As^{V} is desirable, its determination suffers from high detection limits. Additionally, the accuracy and precision for the direct determination of As^{V} by FI-HG-AAS in acid digests of plant samples was about 20–40% lower compared to the target values[20]. Consequently, quantitative pre-reduction of As^{V} to As^{III} is required to reach accurate results and optimum sensitivity. The most popular pre-reductant is KI with ascorbic acid to prevent iodide oxidation by air[20]. Various concentrations of KI (0–9%, w/v) with 5% w/v ascorbic acid were tested. It was found that as signal intensities increased with increasing amounts of KI: ascorbic acid, and was stable with KI: ascorbic acid greater than 1% (w/v) (Fig. 1e). The KI: ascorbic acid concentration of 3% (w/v) was however chosen to make sure that KI/ascorbic acid

Table 3 Analytical performance of FI-HG-AAS and SWCSV method.

Characteristics	FI-HG-AAS		SWCSV	
	As^{III}	TAs_{inorg}	As^{III}	TAs_{inorg}
Linear regression	$y = 0.2694x - 0.0012$	$y = 0.2462x + 0.1254$	$y = 0.6821x + 1.1364$	$y = 0.3818x + 0.4488$
Linear range (μg/l)	1000–20 000	1000–20 000	10–70	10–1000
Correlation coeff. r^2	0.9985	0.9955	0.9942	0.9987
Detection limit (μg/l)	20.0	30.0	0.5	0.4
Quant. limit (μg/l)	70.0	100.0	1.6	1.5
Precision (% RSD)	1.9	3.6	4.0	4.9

concentration is sufficient for the quantitative pre-reduction of As^{V} to As^{III}.

Effect of reducing time

To determine the time required to quantitatively reduce As^{V} to As^{III}, the reduction time was varied from 0–75 min with a sample containing 10 μg/l As^{V} and 3% (w/v) KI/ascorbic acid. It was found that arsenic response was stable for reduction times greater than 30 min (Fig. 1f). Thus the reduction time of 45 min was chosen to allow quantitative reduction of As^{V} to As^{III} in the sample solution.

Moreover, with the optimized KI/ascorbic acid concentration, the efficiency of reduction of As^{V} to As^{III} was evaluated by adding pre-reductant to various concentrations of As^{V} standard solution (2, 4, 6, 8, 10, 15, 20 μg/l) and the % recoveries of As^{V} were observed. It was found that As^{V} can be converted to As^{III} almost 100% or the reduction of As^{V} to As^{III} is quantitative. It is important to note however that the efficiency of the conversion of As^{V} to As^{III} was gradually decreased with increasing of As^{V} concentration. An As^{V} concentration of less than 20 μg/l is therefore recommended for total-As determinations.

Analytical performance of the FI-HG-AAS method

Calibration curves of peak area versus concentration under the optimum conditions revealed a linear range within 1–20 μg/l with a correlation coefficient (r^2) greater than 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on 3σ/m and 10σ/m, respectively, where σ is the standard deviation of 10 measurements of blank signal and m is the slope of the calibration graph. The above procedure was repeated for the detection and quantification limit of As^{III} and total water-soluble inorganic arsenic (TAs_{inorg}). The analytical performance of FI-HG-AAS method for speciation of As^{III} and As^{V} is summarized in Table 3. The accuracy of method was evaluated by analysing three replicates of reference material (CTA-VTL-2) with a certified total arsenic (TAs) concentration of 0.969 ± 0.072 μg/g. The result was 0.961 ± 0.061 μg/g which is in good agreement with the certified value (t-test, 95% confidence level). For inorganic arsenic speciation, however, no certified value of concentration has been provided for arsenic species. The recovery value of inorganic species was therefore quantified by spiking 1, 10, and 20 μg/l of As^{III} and As^{V} to samples after digested by acid. As shown in Table 4, the recovery values for As^{III} and As^{V} were in good agreement with the value of spiked samples (percentage of recovery 90–115%).

Optimization of CSV parameters

Several parameters of CSV for the determination of As were optimized. First of all, differential

Table 4 Experimental recoveries (Rec) for arsenic determination by FI-HG-AAS and SWCSV in lemongrass and turmeric spiked with 1, 10, 20, and 30 μg/l of as^{III} and as^{V}. (ND = non-detected.).

Analytes	FI-HG-AAS				SWCSV			
	As^{III} (μg/l)	Rec (%)	As^{V} (μg/l)	Rec (%)	As^{III} (μg/l)	Rec (%)	As^{V} (μg/l)	Rec (%)
Sample	1.0	–	0.6	–	ND	–	ND	–
Sample + 1.0 μg/l	1.9	90.0	1.7	110.0	–	–	–	–
Sample + 10.0 μg/l	12.5	115.0	10.7	101.0	–	–	–	–
Sample + 20.0 μg/l	22.9	109.5	19.2	93.0	19.6	98.0	18.7	93.5
Sample + 30.0 μg/l	–	–	–	–	28.1	93.7	28.4	94.7

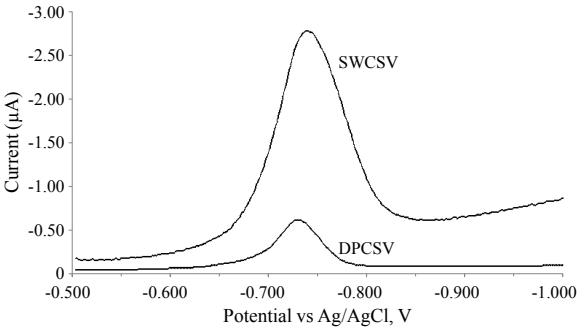

Fig. 2 A comparison between SWCSV and DPCSV modes by using 10 μg/l As^{III} standard solution, 45 mg/l Cu^{II} and 2 M HCl.

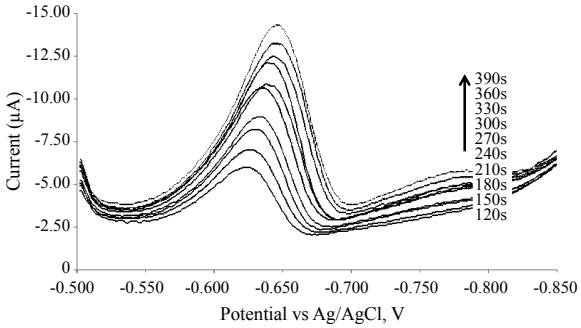

Fig. 3 Effect of deposition time varied from 30–390 s for the SWCSV determination of 20 μg/l As^{V} in HCl 2 M, 3 mM $S_2O_3^{2-}$ and 600 mg/l Cu^{II}.

pulse and square wave mode (DPCSV and SWCSV) were compared (Fig. 2). A square wave mode was found to be about 5 times higher sensitivity than differential pulse mode and thus was chosen for all experiments. In addition, peak area was found to be about 4 times lower sensitivity than that from peak height. Hence peak height was measured throughout all experiments for quantification. Chemical and physical parameters were optimized to obtain the best analytical performance of the CSV system.

Effect of deposition potential

In As^{III} determination, SWCSV deposition potential was varied from −0.30 to −0.50 V. With 20 μg/l As concentration and 45 mg/l Cu^{II}, initially peak response increased, reached maximum at −0.38 V and then decreased. The result was in good agreement with the result by Ferreira and Barros[21]. The deposition potential of −0.38 V was thus chosen for further study. In case of TAs_{inorg} determination, initially the same deposition potential as in the determination of As^{III} was adopted. It was found that in the presence of thiosulphate reducing agent and high concentration of Cu^{II} (600 mg/l), peak current was decreased. SWCSV deposition potential investigation was therefore repeated by varying the potential from −0.36 to −0.42 V. For 20 μg/l As concentration, 600 mg/l Cu^{II} and 3 mM thiosulphate, the deposition potential of −0.39 V was found to give the best signal and was used for TAs_{inorg} determination.

Effect of deposition time

To increase the peak current and improve the method sensitivity, the influence of deposition time was also investigated. For As^{III} determination with the concentration of 20 μg/l and 45 mg/l Cu^{II}, the deposition time was varied between 0 and 240 s. Up to 30 s, the peak was sharp and symmetrical and its height increased with time. Longer deposition time (> 30 s) causes broader peak with less height. Thus it is consistent with previous results by Ferreira and Barros that the higher the Cu^{II} concentration, the lower the accumulation time. Consequently, 30 s was considered optimum for further As^{III} determination. In case of TAs_{inorg} with 10 ml sample containing 20 μg/l As^{V}, 600 mg/l Cu^{II} and 3 mM thiosulphate, the deposition time was varied from 30–390 s. As shown in Fig. 3, the peak current increases continuously with the increase in deposition time, which is different from that in the determination of As^{III}. The deposition time of 240 s was chosen here for routine analysis to keep the time of analysis relatively short.

Effect of HCl concentration

Several previous studies found that chloride plays an important role in the process of deposition of an intermetallic compound (Cu_xAs_y), probably through stabilization of the Cu^{II} formed at the HMDE by complex formation with chloride[21]. The concentrations of HCl as supporting electrolyte were therefore examined from 0–5 M. As shown in Fig. 4, the best result was obtained with 2.0 M HCl and this concentration was used throughout further experiments.

Effect of Cu^{II} concentration

Since arsenic cannot be electrolytically deposited directly onto a Hg electrode, it needs to form an intermetallic compound (Cu_xAs_y). For selective determination of As^{III}, the concentration of Cu^{II} was varied between 0 and 120 mg/l. For 20 μg/l As^{III}, the peak height increased up to 45 mg/l Cu^{II}. Greater concentrations of Cu^{II} from 45 mg/l to

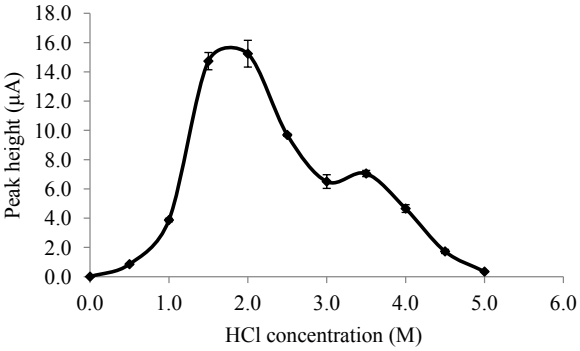

Fig. 4 Effect of HCl concentration with As^{III} 20 μg/l and Cu^{II} 45 mg/l.

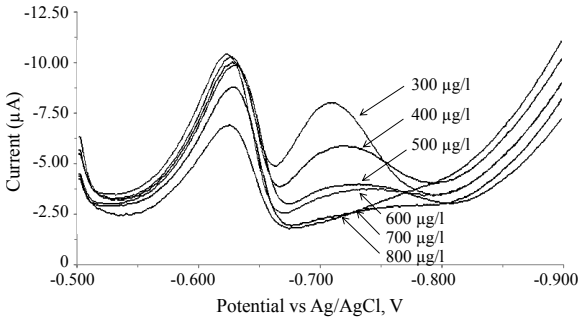

Fig. 5 Effect of Cu^{II} concentration on SWCSV determination of 20 μg/l of As^{V} in 2 M HCl with 2 mM $S_2O_3^{2-}$. Only Cu^{II} concentrations of 300, 400, 500, 600, 700, and 800 mg/l or ppm are shown.

90 mg/l result in a broad and large single peak with decreasing peak height. Beyond these concentrations, the peak disappeared. These types of behaviour were similar to those found by others [17,21,22] which demonstrate the formation of an intermetallic compound with a different Cu:As ratio [16]. A Cu^{II} concentration of 45 mg/l was therefore chosen for As^{III} determination.

For TAs_{inorg} determination, initially the same conditions previously optimized for the determination of As^{III} were adopted (2 M HCl, 45 mg/l Cu^{II}), with As^{V} instead of As^{III}. It was found that, in the presence of thiosulphate, Cu^{II} was less efficient in promoting the deposition of As at Hg electrode which required the use of a much higher concentration of Cu^{II}. The tests with 100–800 mg/l revealed the maximum peak height with 600 mg/l of Cu^{II} (Fig. 5), hence this concentration was selected for further investigation. With the concentration of Cu^{II} lower than 600 mg/l two peaks were obtained, whereas with the concentration higher than 600 mg/l the height of the single peak decreased.

Effect of reducing agent and reducing time

In general, the determination of total inorganic arsenic by using electrometric techniques requires a preliminary step to reduce As^{V} to As^{III}, followed by the total As^{III} determination. Several reducing agents, such as hydroxylamine, oxalic acid, hydrazinium dichloride, KI, bromidic acid, potassium disulphite, Na_2SO_4 and sodium thiosulphate, have been tested to reduce As^{V} to As^{III} but sodium thiosulphate [17] was found to be more convenient, and was selected for this work.

With various concentrations of sodium thiosulphate (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.0, 3.2, 3.6, and 4.0 mM) to quantitatively reduce As^{V} to As^{III}, the peak height increased for concentrations of thiosulphate up to 3.0 mM and then decreased. A concentration of 3.0 mM thiosulphate was therefore chosen.

To determine the time required to quantitatively reduce As^{V} to As^{III}, the reduction time was varied from 5–30 min after the addition of 3 mM thiosulphate to a sample containing 20 μg/l As^{V}. The minimum time required to obtain a reasonable voltammogram with 3 mM thiosulphate was found to be 10 min. Additionally, As response was stable for reduction time greater than 10 min. Thus the reduction time of 10 min was chosen to allow quantitative reduction of As^{V} to As^{III} in the 10 ml sample solution.

Efficiency of As^{V} to As^{III} conversion

To evaluate the efficiency of reduction of As^{V} to As^{III}, three compositions of 20 μg/l total As solution of As^{III}/As^{V} ratios (20/0, 10/10, and 0/20 μg/l) were tested. As can be seen in Fig. 6, the shapes and heights of the peaks are very similar (a 2% RSD of the mean of three measurements), and it can be concluded that the reduction of As^{V} to As^{III} is quantitative.

Analytical performance of the SWCSV method

Calibration curves of SWCSV resulted in the linear range of 10–100 μg/l with correlation coefficient (r^2) greater than 0.99. The above optimized SWCSV was followed for the detection and quantification limit of As^{III} and total water-soluble inorganic arsenic (TAs_{inorg}) to reach the analytical performance of SWCSV method for speciation of As^{III} and As^{V} as given in Table 3.

The accuracy of the method was evaluated by analysing the reference material (CTA-VTL-2). To-

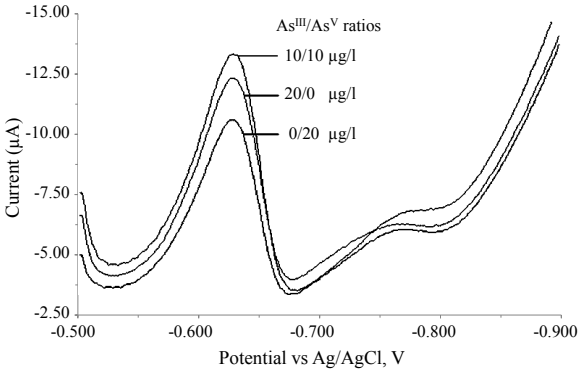

Fig. 6 Efficiency of As^{V} to As^{III} conversion for SWCSV analysis of 20 µg/l of total arsenic (As^{III}+As^{V}) in 2 M HCl, 3 mM $S_2O_3^{2-}$ and 600 mg/l Cu^{II}.

tal arsenic (TAs) concentration was found to be 0.949 ± 0.021 µg/g, in reasonable agreement with the certified value of 0.969 ± 0.072 µg/g. In the case of inorganic arsenic speciation, there is no certified value of concentration for arsenic species. The recovery values of inorganic species were then quantified by spiking 20, and 30 µg/l of As^{III} and As^{V} to samples after acid digestion. The recovery values for As^{III} and As^{V} were found to be corresponding with those of spiked samples (Table 4).

Method comparison for plant sample applications

To compare FI-HG-AAS and SWCSV methods, external calibration and standard addition need to be investigated first (FI-HG-AAS: $n = 3$, standard concentrations 0–8 µg/l; SWCSV: $n = 3$, standard concentrations 0–30 µg/l). Significant differences were observed between the slopes of the external calibration and the standard addition calibration lines (t-test, $p < 0.05$). The standard addition method was therefore used to determine the arsenic in samples to minimize the matrix effect. The method was then applied to determination of As species in lemongrass and turmeric collected from arsenic contaminated area, Ron Phibun subdistrict, Ron Phibun district, Nakhon Si Thammarat province. The content of different arsenic species in the aforementioned lemongrass and turmeric are summarized in Table 5 and Table 6, respectively. There is a significant difference of the capabilities of the two methods to measure inorganic arsenic species. The SWCSV method showed poor sensitivity for trace amount of inorganic arsenic species detections in samples while HG-AAS showed excellent sensitivity for both arsenic species. SWCSV was unable to detect any inorganic species (As^{III} and As^{V}) in either types of lemongrass or turmeric because of its high limit of detection (> 0.4 µg/l for both As species). There was however agreement (t-test, $p<0.05$), especially for turmeric, between total arsenic values obtained by using FI-HG-AAS and SWCSV techniques in lemongrass and turmeric and certified reference material (CTA-VTL-2).

The comparative study of two methods also found that each method has its own merits and limitations. The method of choice will depend on instrument availability, sensitivity and running cost. FI-HG-AAS is well known to be a popular method of As speciation because of its sensitivity and selectivity

Table 5 Arsenic speciation in lemongrass (µg/g).

Villages	Mode	As^{III}	As^{V}	TAs_{inorg} [a]	TAs [b]
M1/1	I[c]	0.026	0.114	0.140	0.238
	II[d]	ND	ND	ND	0.351
M1/2	I	0.020	0.102	0.122	0.226
	II	ND	ND	ND	0.289
M1/3	I	0.031	0.092	0.123	0.316
	II	ND	ND	ND	0.294
M1/4	I	0.038	0.213	0.251	0.304
	II	ND	ND	ND	0.333
M1/5	I	0.036	0.093	0.129	0.247
	II	ND	ND	ND	0.339
M2/1	I	0.034	0.091	0.125	0.381
	II	ND	ND	ND	0.348
M2/2	I	0.038	0.058	0.096	0.231
	II	ND	ND	ND	0.152
M2/3	I	0.049	0.065	0.114	0.301
	II	ND	ND	ND	0.331
M2/4	I	0.385	0.061	0.446	0.979
	II	0.356	0.074	0.430	1.078
M2/5	I	0.040	0.068	0.108	0.465
	II	ND	ND	ND	0.437
M13/1	I	0.022	0.094	0.116	0.339
	II	ND	ND	ND	0.368
M13/2	I	0.023	0.120	0.143	0.281
	II	ND	ND	ND	0.258
M13/3	I	0.010	0.106	0.116	0.148
	II	ND	ND	ND	0.108
M13/4	I	0.036	0.066	0.102	0.175
	II	ND	ND	ND	0.194
M13/5	I	0.022	0.114	0.136	0.197
	II	ND	ND	ND	0.190

[a] Total water-soluble inorganic arsenic ($As^{III} + As^{V}$) extracted by water.
[b] Total arsenic was determined by digesting samples with a $HNO_3/HClO_4/H_2SO_4$ mixture at 100 °C. $n = 3$, SD = 0.000–0.038.
[c] I = FI-HG-AAS;
[d] II = SWCSV.

Table 6 Arsenic speciation in turmeric (μg/g).

Villages	Mode	As^{III}	As^{V}	TAs_{inorg} [a]	TAs [b]
M1/1	I[c]	0.041	0.064	0.105	0.601
	II[d]	ND	ND	ND	0.528
M1/2	I	0.026	0.065	0.091	0.365
	II	ND	ND	ND	0.341
M1/3	I	0.025	0.086	0.111	0.290
	II	ND	ND	ND	0.334
M1/4	I	0.023	0.105	0.128	0.623
	II	ND	ND	ND	0.633
M1/5	I	0.053	0.157	0.210	0.660
	II	ND	ND	ND	0.648
M2/1	I	0.020	0.144	0.164	0.468
	II	ND	ND	ND	0.482
M2/2	I	0.162	0.144	0.306	0.997
	II	ND	ND	ND	0.908
M2/3	I	0.026	0.059	0.085	0.563
	II	ND	ND	ND	0.566
M2/4	I	0.075	0.076	0.151	0.557
	II	ND	ND	ND	0.556
M2/5	I	0.021	0.130	0.151	1.384
	II	ND	ND	ND	1.608
M13/1	I	0.024	0.066	0.090	0.497
	II	ND	ND	ND	0.454
M13/2	I	0.026	0.090	0.116	1.077
	II	ND	ND	ND	1.181
M13/3	I	0.088	0.199	0.287	0.597
	II	ND	ND	ND	0.570
M13/4	I	0.038	0.088	0.126	0.396
	II	ND	ND	ND	0.375
M13/5	I	0.331	0.146	0.477	1.873
	II	0.394	0.262	0.656	1.931

[a] Total water-soluble inorganic arsenic ($As^{III} + As^{V}$) extracted by water.

[b] Total arsenic was determined by digesting samples with a $HNO_3/HClO_4/H_2SO_4$ mixture at 100 °C. $n = 3$, SD = 0.000–0.189.

[c] I = FI-HG-AAS;

[d] II = SWCSV.

but suffers from high costs and laborious sample preparation. Moreover, FI-HG-AAS has limited linearity which can hardly go up to 50 μg/l and it is recommended for low level analytes[15]. SWCSV is a simple, new, cheap, selective, and less laborious technique but limited by its poor sensitivity (high LOD and LOQ). Despite limitations, its linear range can go up 100 μg/l, in strong consistency with previous investigations by Greulach and Henze[23]. It can be stated here that SWCSV is considered to be a promising and alternative method for inorganic arsenic speciation in organism samples but the samples need to contain enough arsenic to suit its LOD values.

CONCLUSIONS

The performance of flow injection-hydride generation-atomic absorption spectrophotometry (FI-HG-AAS) and square wave cathodic stripping voltammetry (SWCSV) have been compared for the speciation of inorganic arsenic species in lemongrass and turmeric. It was found that each method has its own merits and limitations. FI-HG-AAS represents a useful technique for inorganic arsenic speciation, with good performance results (LOD, LOQ, precision, accuracy, and sensitivity) but suffers with high costs and laborious sample preparation. Moreover, HG-AAS has limited linearity to the concentration of only 50 μg/l and it is therefore recommended for analyte with low concentrations. SWCSV provides simplicity, modernity, cost effectiveness, selectivity and labour-saving quality with the limitation of high LOD and LOQ. It can also provide wider linear range up to 100 μg/l together with the benefits of much shorter warm up time than FI-HG-AAS. SWCSV is a promising alternative method for inorganic speciation in organic sample if the arsenic content is higher than its LOD. In the application of both techniques to plant samples, water is an effective extractant for inorganic species with the capability to extract more than 94% of total water-soluble arsenic. The results of total arsenic values by both techniques were in strong agreement with certified reference material (CTA-VTL-2). The main inorganic arsenic species found in the plant samples was As^{V}. As contents in both samples do not exceed the food safety limits of Thailand and several countries.

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