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THESIS

INORGANIC ARSENIC SPECIATION BY A DETECTOR TUBE AND HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

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A Thesis Submitted in Partial Fulfillment of
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Arsenic toxicity is a problem worldwide and in Thailand. It was first recognized in Ron Piboon District, Nakhon Si Thammarat Province. In this study, inorganic arsenic speciation in water samples based on detector tube method and hydride generation atomic absorption spectrometry were developed.

A detector tube containing a new color complex of 2-(5′-chloro-2′-pyridylazo)-5-aminoanisolepalladium(II) chloride as a sensing materials coated on silica gel was prepared. The method based on conversion of arsenic in water to arsine which the color of the sensing materials changed from blue to yellow and the length of color change was proportional to concentration of arsine. Total inorganic As(III) and As(V) were changed to arsine using Zn and sulphamic acid while arsenic(III) was changed to arsine using $NaBH_4$. The linear range of 5 – 25 ppb for both As(III) and total inorganic arsenic with coefficient of determination of 0.9913 and 0.9911, respectively, were obtained. The precision of arsenic determination at 10 ppb (n=6) was 13.07% for As(III), and at 5 ppb and 20 ppb (n=10) were 8.85%, 11.05% for total arsenic.

In hydride generation atomic absorption spectrometry technique, citric acid was found to be a better selective medium for As(III) determination when compared with tris-(hydroxymethyl)aminomethane hydrochloride. Therefore, determination of As(III) was performed in citric acid. The total arsenic was determined in KI/HCl medium. The linear range of 1- 20 ppb for As(III) and As(V) with coefficient of determination of 0.9931 and 0.9972, respectively. The precision of arsenic determination at 5 ppb, 10 ppb and 20 ppb (n=10) were 5.38%, 1.96% and 3.57% for As(III), and 3.05%, 1.23% and 1.24, for total arsenic. The developed method has been successfully applied to determine arsenic(III) and arsenic(V) in well water samples collected in Nakorn Si Thamarat province, Thailand. Of ten samples, four samples contained total arsenic higher than 10 ppb.

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Supaporn Srisupap

July 2014

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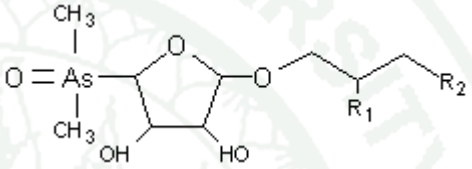
INTRODUCTION

The arsenic in the groundwater is of natural origin, and is released from the sediment into the groundwater, owing to the anoxic conditions of the subsurface. In Thailand, arsenic was found in Ronpiboon district Nakhon Si Thammarat Province in 1987, and the dissolved arsenic in the Chao Phraya River is suspected of containing high levels of naturally occurring arsenic, but has not been a public health problem owing to the use of bottled water. (Siripitayakunkit *et al.* 2003)

Table 1 Arsenical of environmental and biological importance Burguera and Burguera (1997).

Compound	Formula
Arsenious acid ; Arsenites ; As(III)[a]	$HAsO_2$
Arsenic acid ; Arsenates ; As(V)[a]	H_3AsO_4
Monomethylarsonic acid ; (MMAA)[a]	$H_2(CH_3)AsO_3$
Dimethylarsenic acid ; (DMAA)[a]	$H(CH_3)_2AsO_2$
Trimethylarsineoxide ; (TMAO)[a]	$(CH_3)_3As^+O^-$
Tetramethylarsonium ion	$(CH_3)_4As^+$

Table 1 (Continued)

Compound	Formula
Arsenobetaine ; AsB	$(CH_3)_3As+CH_2COOH$
Arsenobetaine ; AsC	$(CH_3)_3As^+CH_2CH_2OH$
Arsenolipids ; AsL	$(CH_3)_3As^+CH_2CHOHCOOH$ (trimethylarsoniumlactate)
Arsenosugars, AsS)[b]	

[a] Compounds forming gaseous species: As(III) and As(V) form AsH_3 (B.P. = -55°C); MMAA forms monomethylarsine (CH_3AsH_2 with B.P.=26°C); DMAA forms dimethylarsine ($(CH_3)_2AsH$ with B.P.=36°C); TMAO form trimethylarsine ($(CH_3)_3As$).

[b] R_1 = OH, R_2 = OH (Giant clams and algae); R_1 = OH, R_2 = SO_3H (Marcoalgae); R_1 = NH_2, R_2 = SO_3H (Brown algae)

Table 2 Contamination of drinking-water by arsenic in various regions of South-East Asia. (IARC monographs Vol. 84. 2004)

Country	Area/Population	Sample	Level of Arsenic (range, µg/L)	Source of arsenic	Reference
Taiwan	South-western Blackfoot	13 artesian well-water	250-960	Natural	Blackwell *et al.* (1961)
	disease-endemic area	34 artesian well-water	350-1100	Natural	Chen *et al.* (1962)
	(Peimen,Hsuehchia,Putai	11 artesian well-water	340-896	Natural	Yeh (1963
	Ichu)	97 artesian well-water	10-1100	Natural	Kuo (1968)
	North-eastern endemic area of chronic arsenic poisoning (Chuangwei,Wuchieh, Chiaohsi,Tungshan)	3901 well-water	<0.15-3590	Natural	Chiou *et al.* (2001)
	Taiwan (314 townships)	83 656 well-water	<10->1000	Natural	Lo (1975)

Table 2 (Continued)

Country	Area/Population	Sample	Level of Arsenic (range, µg/L)	Source of arsenic	Reference
Thailand	Nakornsrihammarat Province	surface water	<0.5-583	Arsenopeyrite	Williams *et al.* (1996)
			<0.528.4As^{III}	wastes	
		shallowe water	1.25-5114		
			<0.5-125 As^{III}		
		surface water	4.8-583	Mining	Choprapawon & Porapakkham (2001)
		River	541-583		
China	Inner Mongolia	497well-water(Huhhot)	<10-1860	Natural	Ma *et al.* (1996); Luo *et al.* (1997
		9733 well-water(Bamen)	<50-890	Natural	Ma *et al.* (1996)

Table 2 (Continued)

Country	Area/Population	Sample	Level of Arsenic (range, µg/L)	Source of arsenic	Reference
	Xinliang	well-water in 15 village (Tinguei)	50-850	Natural	Wang (1996)
	Shanxi	2373 well-water in 129 (Datong, Jinzhong)	<50-4440	Natural	Sun *et al.* (2001)
Japan	Fukuoka	67 well-water	1-293 1–220 As^{V} 15–70 As^{III}	Natural	Kondo *et al.* (1999)
	Sendai		1-35		
	Takatsuki		3-60		
	Kumamoto		5-66		

Table 2 (Continued)

Country	Area/Population	Sample	Level of Arsenic (range, µg/L)	Source of arsenic	Reference
Viet Nam	Red River Basin	68 tubewells,	1–3050 (72% > 10 µg/L)	Natural	Berg *et al.*(2001)
		8 treatment plants	11–190		

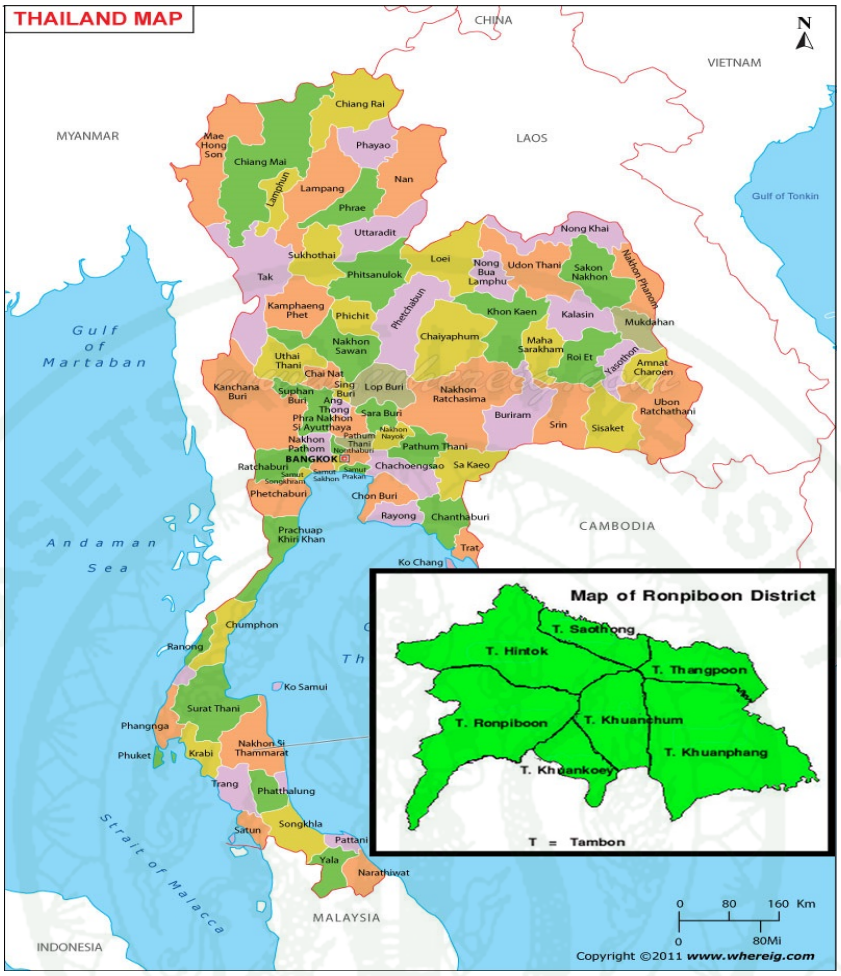


Figure 1 The arsenic polluted areas in Thailand.
(Available from : www.whereig.com/thailand/map-political.html)

Sigrist *et al.* (2011) reported that inorganic As(III) and As(V) forms are the most important species released from mineral dissolution and toxicity of arsenite is greater than that of arsenate. Therefore, there is concern on the determination of speciation of arsenic. The increase of the toxicological and epidemiologic knowledge about arsenic and its species in addition to the advances of the analytical techniques have caused the reduction of the guideline value recommended by the World Health Organization (WHO, 1993) from 50 to 10 μgL^{-1}.

This limit of 10 μgL^{-1} was also set by the United States Environmental Protection Agency (US-EPA), the European Community (EC) and recently by Argentina.

Speciation of inorganic arsenic can be done by both chromatographic methods and nonchromatographic methods. Chromatographic methods include liquid chromatography interfaced with various element specific detectors. The common detectors for this purpose are graphite furnace-atomic absorption (AAS), inductively coupled plasma-mass spectrometry (ICP-MS).

Nonchromatographic methods include a detector tube method, hydride generation on ICP-MS, hydride generation coupled with atomic absorption spectroscopy after selective prereduction treatment. The nonchromatographic method is attractive, particularly for inorganic arsenic speciation as it is less time consuming.

The objectives of this study are to develop nonchromatographic methods which are hydride generation coupled with atomic absorption spectroscopy (HG-AAS) methods and to determine inorganic arsenic speciation in well water samples. For detector tube method a sensing material, 2-(5′-chloro-2′-pyridylazo)-5-aminoanisolepalladium(II)chloride (Pd(II)-5-Cl-PAAA complex) was synthesized by a new approach and a detector tube was newly designed to be able to detect As(III) and As(V) at the regulation limit. For the HG-AAS, the selective medium for arsenic speciation, concentration of selective medium, pH, reducing agent and flow rate of selective medium and reducing agent will be thoroughly investigated.

OBJECTIVES

1. To develop a new detector tube with a 2-(5′-chloro-2′-pyridylazo)-5-aminoanisolepalladium(II)chloride (Pd(II)-5-Cl-PAAA complex) as a sensing material to detect inorganic As(III) and As(V).

2. To develop hydride generation atomic absorption spectrometry method using different selective media for determination of As(III) and As(V).

3. To apply the developed methods to the determination of inorganic arsenic speciation and concentration in well water samples in Nakhon Si Thammarat Province.

LITERATURE REVIEW

1. Literature reviews of arsine detector tube methods.

The detector tube technique is based on the change of color due to the chemical reaction between the chemical gas and the chemical reagent in the tube which is usually used for measurement of chemical gas quantity in the atmosphere. It consists of a glass tube filled with the supporting material such as silica gel, alumina, ground glass, pumic or resin which is impregnated with an indicating chemicals which produces a color change when the sample gas passing through it and the length of stain is measured against a calibration scale. However, all of detector tube methods except HACH method detect total arsenic.

Rahman *et al*. (2004) proposed the generation of arsine and its collection on test paper were made using an arsine generation apparatus, as shown in Figure 2.

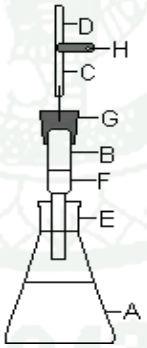


Figure 2 Arsine gas generation apparatus. A, sample container; B, exhaust tube for absorbing hydrogen sulfide gas; C, lower tube; D, upper tube; E, rubber stopper, F, lead acetate-impregnated glass wool; G, rubber stopper; H, stainless-steel clamp.

Potassium iodide, tin(II) chloride, zinc sand in 6M HCl were used a reductant The test paper impregnated with mercury (II) bromide and rosaniline chloride was used as a sensing material and placed between the lower tube (C) and upper (D).The yellow or brownish yellow color intensity on the test paper was measured by a tristimulus colorimeter and by a visual method. The method was successfully applied to determined arsenic in river, brackish and seawater sample.

Cherukuri and Anjaneyulu (2005) proposed a field method, based on the mercuric-bromide-stain. They used zinc metal and hydrochloric acid to produce the 'nascent' hydrogen (reducing agent). The zinc metal has to be of a definite grain size (20-60 mesh) to ensure that the reaction progresses at a certain rate: not too fast – in order to ensure the maximum yield at the reagent paper, and not too slow – in order to allow the determination to be completed in a practicable time. Additionally, sulphide in the sample was first removed by reacting with cupric chloride in combination with ferric chloride. Arsenic in the sample was converted to arsine by reacting with Zn in HCl or sulfamic acid. The reaction could be described as follows:

$$As_2O_3 + 6\ Zn + 12\ HCl \rightarrow 2\ AsH_3 + 6\ ZnCl_2 + 3\ H_2O \text{ ------ (1)}$$

$$H_3AsO_4 + 4\ Zn + 8\ HCl \rightarrow AsH_3 + 4\ ZnCl_2 + 4\ H_2O \text{ -------- (2)}$$

The arsine gas reacting with the reagent paper impregnated with mercuric-bromide showed a white to yellow to tan to brown spot on the reagent paper depending on the concentration of arsine. The quantification of the arsenic concentration was made by visual comparison with a standard color chart.

Khim *et al.* (2010) developed a test kit for determination of arsenic to use in military field drinking water. They used a silver nitrate paper strip instead of mercuric bromide paper strip. The reduction reaction is as follows :

$$As_2O_3 + 6Zn + 12H^+ \longrightarrow 2AsH_3 + 6Zn^{2+} + 3H_2O$$

The arsine gas then reacts with silver nitrate on paper strip to form a color compound, which is silver arsenide:

$$AsH_3 + 3Ag^+ \longrightarrow Ag_3As + 3H^+$$

The test kit is capable to measure arsenic concentration in water as low as 10 ppb which follows the WHO guideline value for arsenic in drinking water. The method was validated with inductively couple plasma mass spectrometry. The results agree well with ICP-Mass spectrometry.

Tahir *et al.* (2012) used a spectrophotometric method to improve the detection. The method is based on reduction of arsenic to arsine gas by a mixture of zinc, stannous chloride, potassium iodide and hydrochloric acid in a specially designed distillation apparatus. The arsine (AsH_3) is passed through a scrubber containing cotton saturated with lead acetate and then into an absorber tube containing silver diethyldithiocarbamate in pyridine. The arsine forms a red complex with silver diethyldithiocarbamate. The absorbance of the complex was read at 535 nm. The 1 ppb arsenic concentration can be determined.

2. Hydride generation coupled with spectrometric methods.

Among the spectrometric method coupled to hydride generation atomic absorption spectrometry are used most for hydride generation. The reason partly may be due to its cheaper cost than ICP-MS.

Samanta *et al.* (1999) used flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) system for inorganic arsenic speciation and quantification in water and biological sample. Speciation and quantification of arsenic (arsenite, arsenate, MMAA and DMAA) of arsenic in urine was carried out with combined cation and anion – exchange resin columns followed by FI-HG-AAS determination. DMAA is absorbed on cation – exchange column while MMAA and As(V) was absorbed on anion – exchange column. As(III) was not retained by both columns. MMAA was eluted from the column using 0.1M sodium acetate – acetic acid buffer while As(V) was eluted from the column using 1.5M sodium acetate. After appropriate pH adjustment, all solution was injected to FI-HG-AAS to quantify individual species.

Delgado-Andrade *et al.* (2003) reported the method for the determination of the total concentration of arsenic in different foods from south-east Spain to estimate daily dietary intake determined by hydride generation atomic absorption spectrometry. Sample was first digested in nitric acid for 45 min and then in HNO_3-$HClO_4$. Standard addition was used to perform arsenic analysis. The reliability and accuracy of method was confirmed by the use of NEST and CBR – CEC reference materials. The estimated intake of total arsenic in the Spanish food diet was 221 µg As day^{-1}.

Gonza´lez *et al.* (2003) studied a non-chromatographic speciation for determination of water-soluble and phosphate-exchangeable As(III) and As(V) in certified reference materials of coal fly ash and sediments by FIHGAAS. Determination of both oxidation states of As in the extracts could be accomplished following arsine generation under different reaction conditions, (i) selective determination of As(III) in citric acid medium or using soft generation conditions; (ii) determination of total As in each extract using thioglycollic acid as reaction medium or after pre-reduction of As(V) to As(III) with a KI + ascorbic acid mixture. The As(V) content was calculated by difference between both measurements.

Niedzielski and Siepak (2003) presented a comparative description of different methods of determination of arsenic, antimony and selenium. For arsenic method, As (III) ions are oxidized by cerium sulphate to As (V), which takes part in the reaction of molybdenium blue formation and is detected at the wavelength of 825 nm. Germanium interference was removed by co-precipitation with hydride iron oxide or extraction with bromide.

Shi *et al.* (2003) developed the speciation procedure to distinguish between As(III) and As(V) in soils using sequential extraction combined with flow injection hydride generation atomic fluorescence determination. Four extraction systems, namely water, 0.6 M KH_2PO_4, 1% (v/v) HCl solution and 1% (w/v) NaOH solution were used. The arsenite in extract was analyzed by HG-AFS in the medium of 0.1 M citric acid. The total arsenic was determined after on-line reduction of arsenate with L-cysteine. More than 75% of total arsenic can't be extracted by these four systems. This fraction of arsenic has low mobility and is considered safe to human-being.

Korenovska and Suhaj (2004) used GF-AAS to determine As^{3+} and As^{5+} in fish product. After digestion under concentrated HCl, As(III) was extracted into $CHCl_3$ first and then back extracted into HCl prior measurement. Then As(V) was determined in the residual fraction by prereducing to As(III) using hydrazine sulfate and HBr and proceed as As(III) analysis.

Maity *et al.* (2004) observed that arsine formation in acetic, oxalic and citric acid from As(V) was negligible but in citric acid the arsine formed from As(III). The method was validated with respect to three synthetic mixture of As(III) and As(V) since SRM for combined As(III) and As(V) in the same solution was not available. Concomitant mineral matrix of the water samples did not interfere with arsenic

determination. Eight out of ten ground water sample contained more As(III) in the range 54-350 ppb.

Akter *et al.* (2005) compared the performance of CE-UV, HG-AAS and LC-ICP-MS for speciation of arsenic in ground water samples. They concluded that LC-ICP-MS is the best choice for routine analysis of arsenite, arsenate and dimethyl arsenic acid.

Almela et al. (2006) determined total arsenic, inorganic arsenic, lead and cadmium in edible seaweed sold in Spain by flow injection-hydride generation atomic-absorption spectrometry (FI-HG-AAS). For total arsenic, samples were treated by ashing aid suspension and nitric acid. Calibration standard solutions of As(III) were prepared from s reduced standard solution of As(V), using a mixture containing 5% (w/v) KI and 5% (w/v) ascorbic acid as reducing solution. For inorganic arsenic analysis, after reduction by HBr and hydrazine sulphate, the inorganic arsenic was extracted into chloroform and back-extracted into HCl. The inorganic arsenic in the back-extraction phase, they found that inorganic arsenic in H.fusifome was three times higher than Tolerable Daily Intakes (TDI) established by WHO.

Wahed *et al.* (2006) modified routine analysis of arsenic content in drinking-water in Bangladesh by HG-AAS. Before HG-AAS analysis, 1 mL of 5 M HCl and 1 mL of 20% KI were added to a 10-mL water sample for the reduction of As(V) to As(III) arsenic. The KI-treated water samples were introduced into a continuous flow of 1 M HCl and 0.4% $NaBH_4$ (in 0.1% NaOH) in a reaction coil to generate the gaseous hydride by a peristaltic pump. The arsenic gas produced was separated from the liquid phase and entered to absorption cell where its absorbance was read. Of 13,286 well samples, 29% of the samples had concentration below WHO guideline value of 10 µg/L.

Chanthai *et al.* (2007) developed a method for speciation analysis of As(III), As(V) and total arsenic by HG-AAS in thai fruit wines and distilled spirits.

The As(V) was determined after reduction to As(III) by potassium iodide plus ascorbic acid in hydrochloric solution for 1 hour prior to measurement while selective determination of As(III) was performed in citrate buffer pH5. The inorganic As species were determined directly in wine and distilled spirit sample after dilution and ethanol evaporation. The total As was determined after acid digestion using appropriate ratio of the samples and concentrated HNO_3. The arsenic species in wine and distilled spirit samples are more of the inorganic arsenic form.

Ronkart *et al*. (2007) developed HPLC-ICP-MS to accurately determine arsenite (As^{III}), arsenate (As^{V}), mono-methylarsonic acid ($MMAA^{V}$), dimethylarsinic acid ($DMAA^{V}$) and arsenobetaine (AsBet) in different water matrices. The developed method showed a high sensitivity with detection limits for each arsenic species close to 0.4 pg injected. Arsenite and arsenate were the major species found in surface and well waters, but AsBet and $DMAA^{V}$ were found in some surface waters, which has never been reported before, while in some natural mineral waters located in volcanic region, the arsenic content exceeded the maximal admissible arsenic content by European legislation standards and the predominant form was As^{V}.

Heitland and Koster (2008) presented a fast determination of arsenic species and total arsenic in urine by HPLC-ICP-MS. The As(III) stock solution was prepared from an As_2O_3 solution in 2% (v/v) HC1 and the As(V) stock solution from an As_2O_5 solution in 3% (v/v) HNO_3 (Merck). The DMA(V) stock solution was prepared by dissolving 98% (m/m) sodium cacodylate, and the MMA(V) stock solution was made by dissolving 99% (m/m) monosodium acid methane arsonate.

Two case studies were investigated. In the first case, they investigated the effect of seafood consumption on the concentration of different forms of arsenic in urine for different person. A maximum enhancement of total As from 1 up to 2200 pg/L (2000 pg/t for As-B) was observed after a normal fish meal.

The second case describes the exposure of a 7-year-old child to As(III) by inhalation of calcium arsenite powder. Five hours after exposure, the concentrations in the child's urine for As-B, DMA(V), As(III), MMA(V), and As(V) were < 0.1, 189, 304, 229, and 27 pg/t, respectively, and these concentrations were reduced to normal background values after 4 days.

Musil and Matoušek (2008) used thioglycolic acid (TGA) for on line pre-reduction of As(V) to As(III). A set up for on-line pre-reduction by TGA was optimized. Limits of detection were 100 ng l^{-1} for I As(III), 135 ng l^{-1} for I As(V) and 30 to 50 ng l^{-1} for methylated arsenicals.

Shraim *et al.* (2008) developed a method to determine total arsenic in ground water samples collected from various locations in United Arab Emirates. L-cysteine was used as a pre-reducing agent for the reduction of pentavalent arsenic species to the trivalent state before mixing with $NaBH_4$ which acted as the main reducing and hydride generating agent and determined arsine generated by HG-AAS. The accuracy of the method was checked by using a CRM and spike recovery. The recovery was found to be 83% with low standard deviation. The advantage of the method was the low acid condition.

HarkabuSoVá *et al.* (2009) studied inorganic arsenic speciation in the rainbow trout muscle and rice samples using high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS). The measurements were carried out on the wavelength of As 193.76 nm. As mobile phase, 10 mmol/l phosphate buffer K_2HPO_4/KH_2PO_4, pH 6.1 was used. For the hydride generation,

1.4% $NaBH_4$ in 0.1 mol/l NaOH and 6 mol/lHCl was used. An oxidizing agent was provided for by the solution of a 1% concentration $K_2S_2O_8$ in 1% NaOH. Oxidation was done by UV. The rainbow trout muscle was extracted by ultrapure deionized water while trifluoroacetic acid was used for the extraction of rice samples.
An inorganic trivalent arsenic As(III) was determined as the main species in rice and non-toxic arsenobetaine in the rainbow trout.

Shah *et al.* (2010) determined arsenic species in muscle tissue of fish species by extraction of arsenic with chloroform first, then microwave digestion with concentrated $HClO_4$ and $Fe_2(SO_4)_3$ and finally detection with electrothermal atomic absorption spectrometry. Certified reference material DORM-2 was used to check the accuracy of the method. The limit of detection of the method was 0.004 and 0.005 µg/g for As(III) and As(V), respectively.

Tuzen *et al.* (2010) determined inorganic arsenic speciation in water, food and biological samples. As(V) was reduced to As(III) by KI and L(+) ascorbic acid was added to model solution containing 0.25 µg of As(III) and 0.25 µg of As(V) and 5 mol/L HCl. After reduction of As(V) to As(III), pH of solution was adjusted to 7 using 5 mol/L NaOH. The levels of arsenic were determined by hydride generation atomic absorption spectrometry. The level of As(V) is calculated by difference of total arsenic and As(III) concentrations.

Janati et al. (2011) determined cadmium, lead, arsenic and mercury in rice from Iran .Wet digestion of samples was performed by using mixtures of two acids, namely, HNO_3–HCl. The results showed that As was the most abundant of the trace elements in rice with average concentration 51.85 ng g^{-1}. The results obtained after complete digestion of these samples and determination using graphite furnace atomic absorption spectrometry(GFAAS) and hydride generation atomic absorption spectrometry (HGAAS).

Erdoğan *et al.* (2011) determined inorganic arsenic species by hydride generation atomic absorption spectrometry in water samples after pre concentration / separation on nano ZrO_2/B_2O_3 by solid phase extraction. Arsenic(V) in the eluent was reduced to As(III) by the addition of 0.15 g of KI and 0.25 g of L(+) ascorbic acid. Then, the resulting As(III) was determined by applying HGAAS procedure. For the determination of As(V) alone, the above procedure was applied directly except the oxidation procedure. Then, the concentration of As(III) was calculated by subtracting the concentration of As(V) from total arsenic concentration.

Karak *et al.* (2011) investigated the distribution of arsenic in these soil profiles from tea gardens in Karbi-anglong (KA), Cachar (CA) and Karimgani (KG) distrial the state of Assam, India by sequential extraction. The transfer of arsenic from soils to plants might be a key step in the route of As entry into foodstuffs. FI-HG-AAS was used to determine arsine generated from As(III) in 0.1 M citric acid and from total arsenic by on line reduction of As(V) with L-cysteine. Organic matter, amorphous Fe and Mn oxyhydroxides and silicates play a role in arsenic retention in the tea garden soil.

Chakma *et al.* (2012) reported analysis of arsenic in rice and rice straw using flow injection hydride generation atomic absorption spectrophotometer. Rice samples were sun-dried. About 0.95-1g samples were taken separately into digestion tube and 10 mL of 69% concentrated nitric acid and 70% of perchloric acid mixture at the ratio of 5:3 was added. About 0.45 - 0.50g rice straw was weighed after further drying at 60ºC to constant weight. It was taken separately into digestion tube and 7 mL of 69% concentrated nitric acid was added. The concentrations in rice and rice straw were 0.235 ± 0.014 ppm (n = 48) and 1.149 ± 0.119 ppm (n = 51), respectively.

Rasmussen *et al.* (2012) reported the use of a strong anion exchange solid – phase extraction (SPE) to selectively elute inorganic As(V) from digestion samples. The final elutes were pre-reduced with KI, ascorbic acid, 0.1% v/v silicone, 3M HCl before the total arsenic concentration was determined by HG-AAS. The mean recoveries of 101 – 104 % and the limit detection of 0.08 mg kg^{-1} were obtained.
The accuracy of the method was confirmed with HPLC couple to ICPMS. The two sets of results were agree well.

Abdel-Lateef *et al.* (2013) proposed a method for determination of As(III) and As(V) in some sediments and water samples using HG-AAS.Various parameters, i.e. the carrier gas flow rate, sample volume, concentration of reducing agent, sodium borohydride, concentration of HCl were optimized. The accuracy was checked by analysis of a lake sediment reference material (IAEA-SL1).

Doker *et al.* (2013) developed for ultra-trace determination of inorganic arsenic species. Arsenic(III) as pyrrolidinedithiocarbamate complex was selectively adsorbed on 30 mg poly(hydroxyethyl methacrylate) (PHEMA) micro beads, which is simply packed into a micropipette-tip. The eluted As(III) complex by ammonia was determined by graphite furnace atomic absorption spectrometry. The enrichment For As(III) was found to be 86. Total arsenic amount was determined after reduction of arsenic(V) to arsenic(III) by thiourea-HCl system. As(V) concentration was calculated by the difference between As(III) and total arsenic. The method was successfully applied to drinking water, snow and reference water (SEM-2011) samples.

Koesmawati *et al.* (2013) developed the method for total arsenic determination in tuna fish using hydride generation quartz furnace atomic absorption spectrophotometry. They dry ashing method with a mixture of magnesium nitrate and magnesium oxide was found to be better than microwave digestion method. The samples were dissolved by adding 10 ml mixture solution of 2M HCl: 15% KI:

15% ascorbic acid of 5:1:2 v/v/v, then warm for 10 minutes at 40-50^{o}C. The solution was put into polypropylene tube and weighted to 25 g by adding 1.5% HCl. The LOD was 0.15 ng g^{-1} and LOQ was 0.23 ng g^{-1}. HGQF-AAS was found sensitive for determination of total arsenic in tuna fish.

Jeremiah O *et al.* (2013) studies levels of arsenic in home-made brews, spirits, in water and raw materials. Aliquot volumes of 10 ml samples were placed in a specially designed reaction vessel and 6M HCl in added in the HG-AAS. Before analysis, a 4% $NaBH_4$ solution was added to convert organic and inorganic arsenic to volatile arsines and trapped in 15% OV-3 chromasorb WAW-PMCSO. The first arsine to be thermally desorbed was AsH_3, which represents total inorganic arsenic in the sample, was purged into the optical cell via a gas transfer line to the atomizer. The levels of arsenic found to be generally below 0.05 mg/L.

Pan *et al.* (2013) determined arsenic in Panax notoginseng by hydride generation atomic fluorescence spectrometry. They used thiourea, thiourea-ascorbic acid = 1:1, ascorbic acid as pre-reduction agent and found that thiourea with content of 10 g L^{-1} was best. The linear relationship between fluorescence intensity and arsenic concentration is in the range of 0-80 μg L^{-1} with a correlation coefficient of 0.9995. The detection limit was 0.036 μg L^{-1} and the recovery was in the range of 90.7-103.5%.

MATERIALS AND METHODS

Materials

1. Apparatus

1.1 The arsenic speciation analysis was performed using a vapor generation accessory (VGA-77) unit connected to electrothermal temperature (ETC-60) and an atomic absorption spectrometer (Spectra AA880Z, Varian USA).

1.2 IR spectra were recorded on a Perkin Elmer 2000 as KBr (in the frequency range 4000-400 cm^{-1}).

1.3 Centrifugation was carried out on a Universal 32 R (Hettich, Germany).

1.4 Ultra-pure water was obtained from water purification system model Simplicity™ (Millipore, France).

1.5 pH measurements were made on a Sartorius PB-11 (USA).

1.6 Evaporation of a solution was performed on Heidolph Hei-VAP equipped with Hei-VAP ML heating bath (Germany).

1.7 An ultrasonic cleaner model Elma S 10 H Elmasonic (Singen, Germany) was used for mixing a silica gel with Pd(II)5-Cl-PAAA solution.

1.8 The stain length was measured by a digital caliper (Winmax, China).

2. Reagents

2.1 Reagents for preparation of arsenic(III), arsenic(V) standard solution and sample solution.

Stock aqueous solution of 1,000 ppm standard solution of As(III)

1,000 ppm standard solution of As(III) was made up by dissolving 0.132 g of As_2O_3 in minimum amount of NaOH, adjusting pH to 3.5 with HCl and diluting to 100 ml with ultrapure water and 0.3g of hydrazinium sulfate was added to prevent oxidation of arsenic(III) to arsenic(V). Solution with 1,000 ppm of arsenic(III) stored at 4°C was stable for at least 1 month. The required standards were prepared daily by dilution of the stock solutions with ultrapure water.

Stock aqueous solution of 1,000 ppm standard solution of As(V)

1,000 ppm standard solution of As(V) (Cerrtified AAS standard) in 0.5 HNO_3 was obtained from Sigma (Steinheim, Germany).

Well water samples were preserved by on-site acidification with 3M HCl to a pH ≤ 2 and keeping the samples in polypropylene bottle under temperature at 4°C.

2.2 Reagents for detector tube technique.

0.1 M potassium hydrogen phthalate (KHP) buffer solution pH 4 was prepared by dissolving 5.1055 g of potassium hydrogen phthalate in ultrapure water and adjusted to pH_4 with a few drops of NaOH solution and finally made up the volume to 250 ml.

15 % (w/v) sodium borohydride solution was made by dissolving 1.5 g of $NaBH_4$ in 10 ml of 0.1M NaOH. This solution should freshly be prepared every 3 hour because $NaBH_4$ is not stable.

0.128 mM sulphur(II) solution was prepared by dissolving 10.398 mg of sodium sulfide (Na_2S) in ultrapure water to 100 ml.

1.28 mM tin(IV) solution was prepared by dissolving 20.26 mg of tin(IV)oxide (SnO_2) in 0.1M HCl to 100 ml.

0.128 mM bismuth(II) solution was prepared by dissolving 19.495 mg of bismuts(II)nitrate ($Bi(NO_3)_2$) in ultrapure water to 100 ml.

0.128 mM antimony(IV) solution was prepared by pipetting 3.022 mg of antimony(III)chloride (Sb_2O_3) in 7% HCl and diluting to 100 ml with ultrapure water.

0.128 mM ferrous(III) solution was prepared by dissolving 5.30 mg of $FeCl_3$ in ultrapure water to 100 ml.

0.128 mM mercury(II) solution was prepared by dissolving 3.620 mg of mercury(II)chloride ($HgCl_2$) in ultrapure water to 100 ml.

0.128 mM monomethyl arsenic(V) solution was prepared by dissolving 0.3892 mg of disodium methylarsonate hexahydrate ($Na_2AsO_3CH_3.6H_2O$) in ultrapure water to 100 ml.

0.128 mM dimethyl arsenic(V) solution was prepared by dissolving 0.1840 mg of cacodylic acid ($C_2H_7AsO_2$) in ultrapure water to 100 ml.

0.128 mM arsenic(III) solution was prepared by dissolving 2.63 mg of arsenic(III)oxide (As_2O_3) in 0.1M NaOH and diluting to 100 ml with ultrapure water.

0.128 mM arsenic(V) solution was prepared by dissolving 1.892 mg of H_3AsO_4 in ultrapure water to 100 ml.

2.3 Reagents for hydride generation atomic absorption spectrometry technique**.**

100 ppm standard solution of arsenic(III) was prepared by pipetting 2.5 ml of 1000 ppm stock standard solution in 25 ml volumetric flask and a diluting to the mark with deionized water.

100 ppm standard solution of arsenic(V) in 7M HCl was prepared by pipetting 2.5 ml of 1000 ppm stock standard solution in 25 ml volumetric flask and added 17.50 ml of 37% HCl diluting to the mark with deionized water.

0.5% $NaBH_4$ in 0.5% NaOH + 10% KI solution was prepared by dissolving 1.25 g of NaOH, 1.5 g of $NaBH_4$ and 25 g of KI in 250 ml volumetric flask diluting to the mark with deionized water as a reductant for total arsenic

determination. This solution was freshly prepared every 3 hour because $NaBH_4$ was not stable.

0.5% $NaBH_4$ in 0.5% NaOH solution was prepared by dissolving 1.25 g of NaOH, 1.5 g of $NaBH_4$ in 250 ml volumetric flask diluting to the mark with deionized water as a reductant for As(III) determination. This solution was freshly prepared every 3 hour because $NaBH_4$ was not stable.

1.0M Citric acid was prepared by dissolving 16.30 g of $C_6H_8O_7$ in 100 ml volumetric flask and diluting to the mark with deionized water.

5M HCl was prepared by pipetting 83.33 ml of 37% HCl in 200 ml diluting to the mark with deionized water.

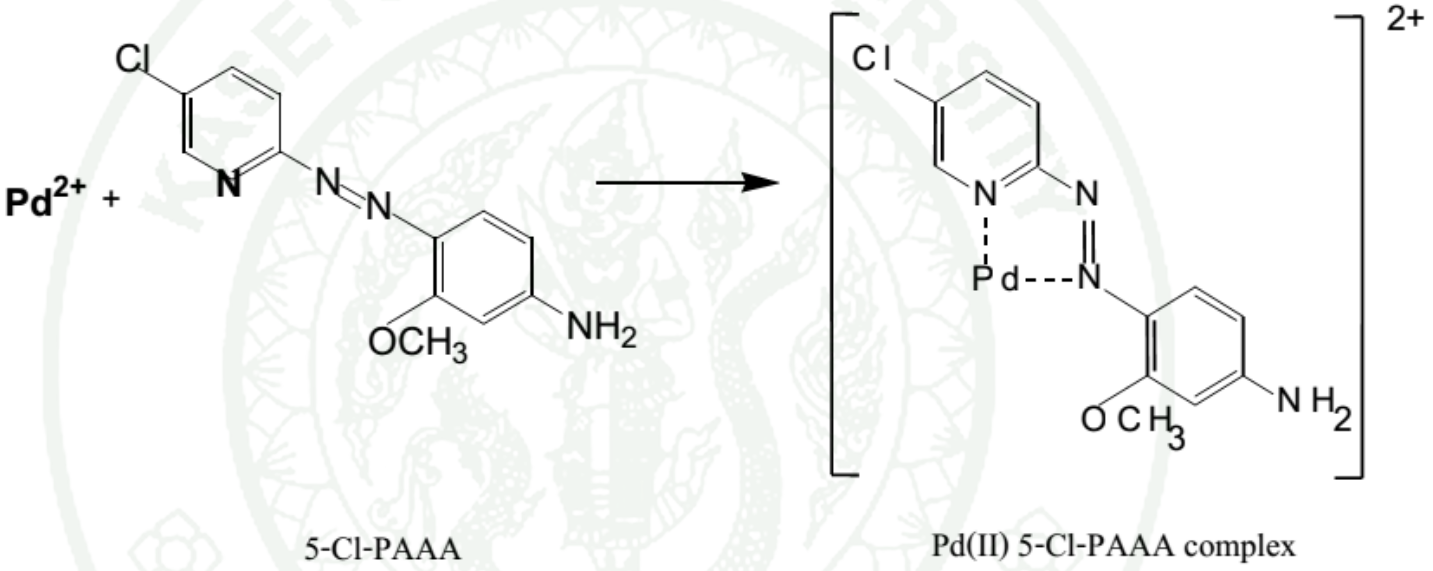
Methods

There are two parts namely part I for development of detector tube for inorganic arsenic speciation and part II development of hydride generation method for inorganic arsenic speciation.

Part 1 Development of a detector tube for determination of inorganic arsenic speciation.

1. The preparation of 2-(5´-chloro-2´- pyridylazo)-5- aminoanisolepalladium(II) chloride (Pd(II)-5-Cl-PAAA complex) as follows:

A solution of 5-Cl-PAAA (10 mg, 0.038 mmol) in 25 ml $CHCl_3$: ethanol (1:1) was dropped with a stirring to a solution of palladium chloride (6.74 mg, 0.038 mmol) in 0.01M HCl 10 ml and ethanol 5 ml. The mixture was reflux at 60 ^{0}C for 1 hour, the solution was evaporated to reduce volume to 20 ml and then the mixture was centrifuged. The crude product was washed with diethyl ether to afford Pd(II)-5-Cl-PAAA complex. Figure 3 shows the reaction of Pd^{2+} with 5-Cl-PAAA.



5-Cl-PAAA = 2 – (5' – chloro-2'-Pyridyazo)

Figure 3 Postulated formula of 2-(5′-chloro-2′-pyridylazo)-5-aminoanisolepalladium(II)chloride.

2. Characterization of 2-(5'-chloro-2'-pyridylazo-5-aminoanisolepalladium(II) chloride.

2-(5'-chloro-2'-pyridylazo-5-aminoanisolepalladium(II) chloride complex was spectroscopic characterized by FT-IR and high resolution mass spectroscopy. 2-(5'-chloro-2'-pyridylazo-5-aminoanisolepalladium(II) chloride complex infrared spectra. Additionally, the complex was also characterized by elemental analysis.

3. Determination of Arsenic(V) using a detector tube.

3.1 Reducing agent study for As(V) determination for detector tube method.

Experiment for Zn powder with acid such as oxalic acid, tris-hydroxylmethyl aminomethane, citric acid and sulphamic acid as a reducing agent and hydrogen generator were studies. The results are shown in Table 5. Zn powder with sulphamic acid was used in the rest of the study.

Method 1: With oxalic acid

The solution containing 40 ml of As(V) 25 ppb, 1.0 g of Zn powder and 2.5g of oxalic acid was added. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 1 hour. The results are shown in Table 5.

Method 2: With tris-(hydroxylmethyl) aminomethane

The solution containing 40 ml of As(V) 25 ppb, 1.0 g of Zn powder and 2.5 g of sulphamic acid was added. The flask was immediately capped with silicone

stopper with a detector tube in the middle of the stopper and stand for 1 hour. The results are shown in Table 5.

Method 3: With citric acid

The solution containing 40 ml of As(V) 25 ppb, 1.0 g of Zn powder and 2.5g of citric acid was added. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 1 hour. The results are shown in Table 5.

Method 4: With sulphamic acid

The solution containing 40 ml of As(V) 25 ppb, 1.0 g of Zn powder and 2.5g of sulphamic acid was added. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 1 hour. The results are shown in Table 5.

3.2 Optimization amount of complex coating on silica gel.

The varying volume solution of 20 ppm 2-(5'-chloro-2'-pyridylazo-5-aminoanisole palladium(II)chloride in dichloromethane (1mg in 50ml) i.e 2ml, 3ml, 4ml and 5ml were used to coating silica. The solution containing 40 ml of As(V) 5 ppb, 2.0g of Zn powder and 3.0g of sulphamic acid were placed in a series of 50 ml flask and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper. The results are shown in Table 6 and Figure 5.

3.3 Optimization amount of Zn powder on reduction of As(V) to As(III).

The effect of varying the amount of Zn powder, i.e 1.0 g, 1.5 g, 2.0 g, 2.5 g and 3.0g on arsenic(V) determination were studied. The solution containing 40 ml of As(V) 25 ppb, 1.0g, 1.5g, 2.0g, 2.5g and 3.0g of Zn powder were placed in a series of 50 ml flask. After thoroughly mixing 2.0g of sulphamic acid were added, and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper. The results are shown in Table 7 and Figure 6.

The coated silica gel was packed into a tube and put in the middle of the stopper. Solution used to test this experiment was as follows:

3.4 Optimization amount of sulphamic acid on reduction of As(V) to As(III).

The effect of varying the amount of sulphamic acid, i.e 2.0, 2.5g, 3.0g and 3.5g on As(V) determination were investigated. The solution containing 40 ml of As(V) 25 ppb, 2.5g of Zn powder were place in a series of 50 ml flask. After thoroughly mixing, 2.0, 2.5g, 3.0g and 3.5g of sulphamic acid were added, respectively and immediately the flask was capped with silicone stopper with a detector tube in the middle of the stopper. The results are shown in Table 8 and Figure 7.

3.5 Optimization of reaction time on reduction of As(V) to As(III).

The effect of varying reaction time on reduction time, i.e 15, 20, 25, 30, 35, 40 and 45 min on arsenic(V) determination were studied. The solution containing 40 ml of arsenic(V) 25 ppb, 2.5g of Zn powder were placed in a series of 50 ml flask. After thoroughly mixing, immediately the flask was capped with silicone stopper with

a detector tube in the middle of the stopper. The results are shown in Table 9 and Figure 8.

3.6 Validation of the method.

To validate the method for As(V) and total As (As(V) and As(III), working range, precision, interference study and comparison of results obtained with HG-AAS were investigated.

3.6.1 Calibration curve.

The solution containing 40 ml of As(V) with concentration of 5, 10, 15, 20 and 25 ppb , 2.5 g of Zn powder and 2.5g of sulphamic acid was added into a series of arsine generation reactors. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute. Each concentration was measured in triplicate. The results of As(V) are shown in Figure 9 and Appendix Figures 2, respectively.

3.6.2 Precision study.

The precision was studied by determination of As(V) concentration at 5 ppb and 20 ppb levels. Ten replicates were investigated at each levels described in section 3.6.1. The results were presented in Table 10.

3.7 Interference studies on the determination of As(V).

3.7.1 Effect of sulphide ion on As(V) detector tube.

The solution containing 20 ml of 20ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.4, 1.2 and 1.6ml of sodium sulfite stock solution

was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute. The results are shown in Table 11.

3.7.2 Effect of tin(IV) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.4, 2.0 and 4.0 ml of tin(IV) stock solution was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute.The results are shown in Table 12.

3.7.3 Effect of bismuth(II) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.4, 0.8 and 1.2 ml of bismuth(II)nitrate stock solution was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute. The results are shown in Table 13.

3.7.4 Effect of antimony(III) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 2.0, 6.0 and 10 ml of antimony(III)chloride stock solution was added into a series of arsine generation reactors. The final volume was

adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute. The results are shown in Table14.

3.7.5 Effect of As(III) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(III), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.02, 0.32 and 0.4 ml of As(III) stock solution was added was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute. The results are shown in Table 15.

3.7.6 Effect of ferrous(III) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.4, 0.2 and 4.0 ml of ferrous(III) stock solution was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minute. The results are shown in Table 16.

3.7.7 Effect of mercury(II)chloride on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 12, 16 and 20 ml of mercury(II)chloride stock solution was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone

stopper with a detector tube in the middle of the stopper and stand for 35 minutes. The results are shown in Table 17.

3.7.8 Effect of disodium methylarsonate hexahydrate, MMAs (V) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.04, 0.12 and 0.2 ml of MMAs(V) stock solution was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minutes. The results are shown in Table 18.

3.7.9 Effect of cacodylic acid, DMAs(V) on As(V) detector tube.

The solution containing 20 ml of 20 ppb As(V), 2.5 g of Zn powder and 2.5g of sulphamic acid and 0, 0.04, 0.2 and 0.4 ml of DMAs(V) stock solution was added into a series of arsine generation reactors. The final volume was adjusted 40 ml with deionized water. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 35 minutes. The results are shown in Table 19.

3.8 As(V) determination of well water samples using detector tube.

Ten well water samples from Ronpibool district is south of Nakorn Sri Thammarat Province, in the southern part of Thailand were investigated for mornitoring of As(V) by detector tube methods (three replication for each sample) as shown in Table 20. The sample preparation was carried out as described in section 2.1. 4. Determination of As(III) using a detector tube.

4.1 The effect of 0.1M potassium hydrogen phthalate (KHP) buffer.

The effect of varying the KHP buffer pH 2, pH 3, pH 4 and pH 5 on As(III) 25 ppb determination were studied. The solution containing 20 ml of As(III) 25 ppb, 5ml of 0.1M potassium hydrogen phthalate (KHP) buffer at pH 2, 3, 4 and 5 were placed into a series of 50 ml flasks. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper. The results are shown in Table 21 and Figure 10. The KHP buffer at pH4 was used in the rest of the study.

4.2 Optimization of concentration of $NaBH_4$ solution.

The effect of concentration of $NaBH_4$ solution 1%, 5%, 10%, 15% and 20% (w/v) were studied. The solution containing 20 ml of arsenic(III) 25 ppb , 5ml of 0.1M potassium hydrogen phthalate (KHP) buffer at pH4 were placed into a series of 50 ml flasks. After thoroughly mixing immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper. The results are shown in Table 22 and Figure 11. The concentration of $NaBH_4$ solution 15% (w/v) was used in the rest of the study.

4.3 Optimization of detection time of a detector tube.

The effect of reaction time on arsenic(III) determination 5, 10, 12, 15, 18 and 20 minutes were studied. The solution containing 20 ml of arsenic(III) 25 ppb, 5ml of 0.1M potassium hydrogen phthalate (KHP) buffer were placed in a series of 50 ml flask. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with a silicone stopper with a detector tube in the middle of the stopper. The results are shown in Table 23 and Figure 12. The detection time 15 minute was used in the rest of the study.

4.4. Validation of the method.

The arsenic(III) standard solution were used for validation of the method in this work. Validation of the method involves linear range, precision, interference study and comparison of results with hydride generation atomic absorption spectrometry.

4.4.1 Calibration curve.

The solution containing 20 ml of varying concentration of arsenic(III) standard solution and 5 ml of KHP buffer solution at pH 4 were added into with concentration of 5, 10, 15, 20 and 25 ppb into a series of arsine generation reactors and then 1 ml of 15% $NaBH_4$ was injected in the reactors. The flask was immediately capped with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. Each concentration was detected in triplicate. The results of As(III) are shown in Figure 13.

4.4.2 Precision.

The precision was studied by determination of arsenic(III) concentration at 10 replicates and 6 replicates was detect using the same conditions as described in 4.4.1. The results are presented in Table 24.

4.5. Interference study.

The interference ions used in the study of effect of interferences in the determination of arsenic(III) at 10 ppb were S(II), Hg(II), Bi(II), Sb(IV), Sn(IV), As(III), As(V), MMAs(V) and DMAs(V). In this study, the detector tube analysis was carried out using the same conditions as describe in 4.4.1. The results of each interference ion study are presented in Tables 25 – 33.

4.5.1 Effect of sulfide ion on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), 0, 0.4, 1.2 and 1.6 ml of sulfide stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjusted to 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. The results are shown in Table 25.

4.5.2 Effect of tin(IV) on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 1.0, 1.4,1.8 and 2.0 ml of tin(IV) stock solution and 5 ml of KHP buffer pH4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjusted to 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minute. The results are shown in Table 26.

4.5.3 Effect of bismuth(II)nitrate on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 0.1, 0.14 and 0.18 ml of bismuth(II)nitrate stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjusted to 20 ml deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 27.

4.5.4 Effect of antimony(III) on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 0.2 and 0.6 of Antimony(III) stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjust to 20 ml 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 28.

4.5.5 Effect of As(V) on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 0.1, 0.14 and 0.18 ml of As(V) stock solution and 5 ml of KHP buffer pH4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjust to 20 ml 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 29.

4.5.6 Effect of ferrous(III) on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 0.2, 1.0 and 2.0 of ferrous stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjust to 20 ml 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 30.

4.5.7 Effect of mercury(II) on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 0.2, 1.0 and 10 ml of mercury(II)chloride stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjust to 20 ml 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 31.

4.5.8 Effect of disodium methylarsonate hexahydrate, MMAs(V) on As(III) detector tube.

The solution containing 10 ml of 20 ppb As(III), and 0, 0.4, 0.6 and 0.8 ml of MMAs(V) stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjusted to 20 ml 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 32.

4.5.9 Effect of cacodylic acid, DMAs(V) on As(III) detector tube.

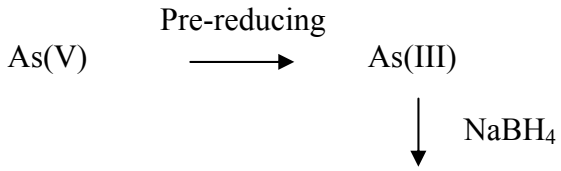
The solution containing 10 ml of 20 ppb As(III), and 0, 0.06, 0.12 and 0.1 ml of DMAs(V) stock solution and 5 ml of KHP buffer pH 4 were placed in a series of hydride generation reactors and the total volume of the reactor was then adjusted to 20 ml 20 ml with deionized water. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask with silicone stopper with a detector tube in the middle of the stopper and detection was made of 15 minutes. The results are shown in Table 33.

4.6 As(III) determination of surface water samples using detector tube.

Ten well water samples from Ronpibool district is south of Nakorn Si Thammarat Province, in the southern part of Thailand were investigated for mornitoring of As(III)) by detector tube and HG-AAS methods (three replication for each sample) as shown in Table 34. The sample preparation was carried out as described in materials and methods section 2.1.

5. Pre-reductant study for As(V) determination for detector tube method.

From preliminary experiments, we found that sodium borohydride method gave higher sensitivity for As(III) than that of Zn method. Therefore, pre-reducing agent (L-cysteine, thiourea, potassium iodide, ascorbic acid and hydroxylamine hydrochloride) were tried to convert As(V) to As(III) for determination of As(V) as Figure 4.



AsH_3

Figure 4 Diagram of pre-reductant study for As(V) determination with sodium borohydride method.

5.1 Experiment for L-cysteine as a pre-reductant.

The solution containing 40 ml of 25 ppb As(V), 0.5g of L-cysteine and 5 ml of KHP buffer pH4 were placed in a 50 ml flasks. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask was capped with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. The results are shown in Table 35.

5.2 Experiments for thiourea as a pre-reductant.

The solution containing 40 ml of 25 ppb As(V), 0.5g of thiourea and 5 ml of KHP buffer pH4 were placed in a 50 ml flasks. After thoroughly mixing, 1ml of 15% $NaBH_4$ was added and immediately capped the flask was capped with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. The results are shown in Table 35.

5.3 Experiment for potassium iodide as a pre-reductant.

The solution containing 40 ml of 25 ppb As(V), 0.5g of potassium iodide and 5 ml of KHP buffer pH4 were place in a 50 ml flasks. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask was capped with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. The results are shown in Table 35.

5.4 Experiment for ascobic acid as a pre-reductant.

The solution containing 40 ml of 25 ppb As(V), 0.5g of ascorbic acid and 5 ml of KHP buffer pH4 were place in a 50 ml flasks. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask was capped with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. The results are shown in Table 35.

5.5 Experiment for hydroxyl ammonium chloride as a pre-reductant.

The solution containing 40 ml of 25 ppb As(V), 0.5g of hydroxyl ammonium chloride and 5 ml of KHP buffer pH 4 were place in a 50 ml flasks. After thoroughly mixing, 1 ml of 15% $NaBH_4$ was added and immediately capped the flask was capped with silicone stopper with a detector tube in the middle of the stopper and stand for 15 minutes. The results are shown in Table 35.

Part 2 : Hydride generation atomic absorption spectrometric method.

A method for determination of inorganic arsenic speciation was developed by using selective acid medium in the hydride generation of arsine from As(III) followed by furnace atomic absorption measurement.

1. Two acid media were chosen for comparative study on As(III) determination by hydride generation furnace atomic absorption method.

They were tris-(hydroxymethyl)-aminomethane hydrochloride and citric acid. A solution mixture containing 15 ppb and 75 ppb of As(III) and As(V), respectively, was used throughout this comparison study.

1.1 Tris-(hydroxymethyl)-aminomethane hydrochloride was studies for acid medium on As(III) determination by hydride generation atomic absorption method.

1.1.1 Effect of pH of 2.5M tris-(hydroxymethyl)-aminomethane hydrochloride was studied.

The pH of 2.5M tris-(hydroxymethyl)-aminomethane hydrochloride was varied from pH 5 - 9 at 2% $NaBH_4$ (%w/v) in 0.02M NaOH. The mean absorbance of 15 ppb As(III) and mixing solution (As(III) 15ppb + As(V) 75 ppb). The results are shown in Figure 14. The percentage difference between As(III) in a pure solution and As(III) in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution.The results are shown in Figure 15.

1.1.2 Effect of $NaBH_4$ on the determination of As(III) in a pure solution and a mixture when 2.5M tris-(hydroxymethyl)-aminomethane hydrochloride used as acid medium.

The effect of sodium tetrahydroborate concentration was varied from 0.5% - 4% at pH 6 of 2.5M tris-(hydroxymethyl)aminomethane hydrochloride. The mean absorbance of 15 ppb As(III) and mixing solution (As(III) 15ppb + As(V) 75 ppb). The results are shown in Figure 16. The percentage difference between As(III) in a pure solution and in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution. The results are shown in Figure 17.

1.1.3 Effect of NaOH on the determination of As(III) in a pure solution and a mixture when 2.5M tris-(hydroxymethyl)-aminomethane hydrochloride used as acid medium.

The effect of sodium hydroxide concentration was varied from 0.02-0.2M at pH 6 of 2.5M tris-(hydroxymethyl)aminomethane hydrochloride. The mean absorbance of 15 ppb As(III) and that of a mixture (As(III) 15ppb + As(V) 75 ppb). The results are shown in Figure 18. The percentage difference between As(III) in pure solution and in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution. The results are shown in Figure 19.

1.2 Citric acid was studied for an selective acid medium on the determination of As(III) by hydride generation atomic absorption method.

1.2.1 Effect of concentration of citric acid.

The effect of concentration of citric acid was varied from 0.1 - 1.5 M at 0.5% $NaBH_4$ in 0.5% NaOH on the mean absorbance of 15 ppb As(III) in a pure solution and in a mixture. The results are shown in Figure 20. The percentage difference between As(III) in a pure solution and in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution. The results are shown in Figure 21.

1.2.2 Effect of sodium tetrahydroborate at 1.0M citric acid.

The effect of sodium tetrahydroborate concentration was varied from 0.5% - 3.5% (%w/v) at 1.0M citric acid on the mean absorbance of 15 ppb As(III) in a pure solution and in a mixture. The results are shown in Figure 22. The percentage difference between As(III) in a pure solution and in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution. The results are shown in Figure 23.

1.2.3 Effect of sodium hydroxide at 1.0M citric acid.

The effect of sodium hydroxide concentration was varied from 0.5-1.0% (%w/v) at 1.0M citric acid. The mean absorbance of 15 ppb As(III) in a pure solution and in a mixture. The results are shown in Figure 24. The percentage difference between As(III) in a pure solution and in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution. The results are shown in Figure 25.

1.3 Calibration curve for As(III) determination by hydride generation atomic absorption.

Hydride generation-atomic absorption spectrometry for As(III) determination of well water samples. In citric acid medium, the calibration curve for As(III) was obtained in the range 1 ppb to 20 ppb. The calibration curve for As(III) was constructed by pipetting 0.5, 2.5, 5.0, 7.5 and 10 ml of 100 ppb As(III) stock solution in a series of 50 ml volumetric flasks and the volume was diluted and diluting to the mark with deionized water. The calibration curve for As(III) by hydride generation atomic absorption is shown in Figure 26.

1.4 Precision study for As(III) determination by hydride generation atomic absorption.

The precision of the method was studied by determination of As(III) concentration at 5 ppb, 10 ppb and 20 ppb and 10 replicates were detected at each concentration. The results are shows in Table 36.

1.5 As(III) determination of well water samples using hydride generation atomic absorption technique.

The method of hydride generation atomic absorption for As(III) was used in the analysis of well water samples collected in Nakorn Si Thammarat as shown in Table 3.

Table 3 Operating condition of the HG-AAS system for As(III).

Method : HG-AAS for Arsenic(III)

Wavelength	193.7 nm
Slit width	0.5 nm
Lamp	Ultra lamp(Varian)
Temperature	925°C
Reductant concentration	0.5% $NaBH_4$ in 0.5% NaOH
Acid concentration	1.0M Citric acid
Reductant flow rate	1 ml min^{-1}
Acid flow rate	1 ml min^{-1}
Sample flow rate	7 ml min^{-1}
Delay time	50 sec

2. As(V) determination by hydride generation-atomic absorption spectrometry.

2.1 Calibration curve for As(V) determination by hydride generation atomic absorption spectrometry.

Hydride generation atomic absorption technique for total arsenic determination in well water samples with KI and HCl medium, the calibration curve for total inorganic arsenic was found to be linear in the range 1ppb to 20 ppb. The calibration curve for As(III) was constructed by pipetting 0.5, 2.5, 5.0,7.5 and 10 ml of 100 ppb As(III) stock solution in a series of 50 ml volumetric flasks. After addition of 17.5 ml of 37% HCl the solution was diluting to the mark with deionized water.

The calibration curve for As(III) by hydride generation atomic absorption is shown in Figure 27.

2.2 Precision study for As(V) determination by hydride generation atomic absorption.

The precision of the method was studied by determination of As(V) concentration at 5 ppb, 10 ppb and 20 ppb and 10 replicates were measured. The results are shows in Table 37.

2.3 Arsenic(V) determination of well water samples using hydride generation atomic absorption technique.

The method of hydride generation atomic absorption for total arsenic was used in the analysis of well water samples collected in Nakorn Si Thammarat as shown in Table 4.

Table 4 Operating conditions of the HG-AAS system for As(V) / total arsenic.

Method : HG-AAS for Total Arsenic

Wavelength	193.7 nm
Slit width	0.5 nm
Lamp	Ultra lamp(Varian)
Temperature	925°C
Reductant concentration	0.5% $NaBH_4$ in 0.5% NaOH and 10% KI
Acid concentration	5 M HCl
Reductant flow rate	1 ml min^{-1}
Acid flow rate	1 ml min^{-1}
Sample flow rate	7 ml min^{-1}
Delay time	50 sec

RESULTS AND DISCUSSION

Part 1: Developed a detector tube for determination arsenic speciation.

1. Preparation of 2-(5′-chloro-2′-pyridylazo)-5-aminoanisolepalladium(II)chloride.

PAAA reacts with Pd(II) ion resulting in a blue black complex of 1:1 ratio of ligand to Pd(II). The results of the elemental analysis and mass spectrum are in good agreement with those required by the proposed formula. Final yield of 41.63% was obtained.

2. Characterization of 2-(5'-chloro-2'-pyridylazo-5-aminoanisolepalladium(II) chloride.

2.1 The FT-IR spectra of 2-(5′-chloro-2′-pyridylazo)-5-aminoanisole-palladium(II) chloride and that of ligand.

The IR spectra of PAAA and its palladium complex were carried out in the range 400 – 4000 cm^{-1} range. The spectral region around 1600 – 1400 cm^{-1} is complicated due to the superimposition of C=C and N=N.

The involvement of C=N band in the ligand (1648 cm^{-1}) was shown by the blue shift to 1634 cm^{-1} in the palladium complex.

2.2 Elemental analysis

Elemental analysis calculated for $C_{12}H_{11}N_4OPdCl_3$: C, 32.75% ; H, 2.51% ; N, 12.73% Found : C, 32.75% ; H, 2.40% ; N, 12.80%.

2.3 High resolution mass spectrometry (micro TOF).

For Pd(II)-5-Cl-PAAA analysis, $[M+Na]^+$: m/z 462.8912.

3. Determination of Arsenic(V) and or total arsenic determination.

3.1 Screening of acids for total arsenic determination.

Experiment for Zn powder with acid such as oxalic acid, citric acid, tris-hydroxylmethyl aminomethane and sulphamic acid as a reducing agent and hydrogen generator were studies. The results are shown in Table 5. Zn powder with sulphamic acid was used in the rest of the study.

Table 5 Screening of acids for total arsenic detection.

Acid	Stain length (cm)
Blank	0
oxalic acid	no gas formation
tris-hydroxylamine aminomethane	gas formation but no color change
citric acid	0.502
sulphamic acid	0.702

3.2 Optimization of volume of a 20 ppm 2-(5'-chloro-2'-pyridylazo-5-aminoanisolepalladium(II)chloride used in coating 1 gm of silica gel.

However, the boundary between unreacted zone and reacted zone is obvious when 4.0 mL of 20 ppm complex solution was used. As shown in Table 6 and Figure 5, the stain length of As(V) concentration increase with decreasing of volume of 20 ppm 2-(5'-chloro-2'-pyridylazo-5-aminoanisolepalladium(II)chloride.Four milliliters of 20 ppm palladium complex was chosen for subsequent studies.

Table 6 Stain length at various milliliter of 20 ppm 2-(5'-chloro-2'-pyridylazo-5-aminoanisole palladium(II)chloride.

Volume (ml)	Stain length (cm.)
2.0	difficult to see the boundary
3.0	0.285
4.0	0.264
5.0	0.251

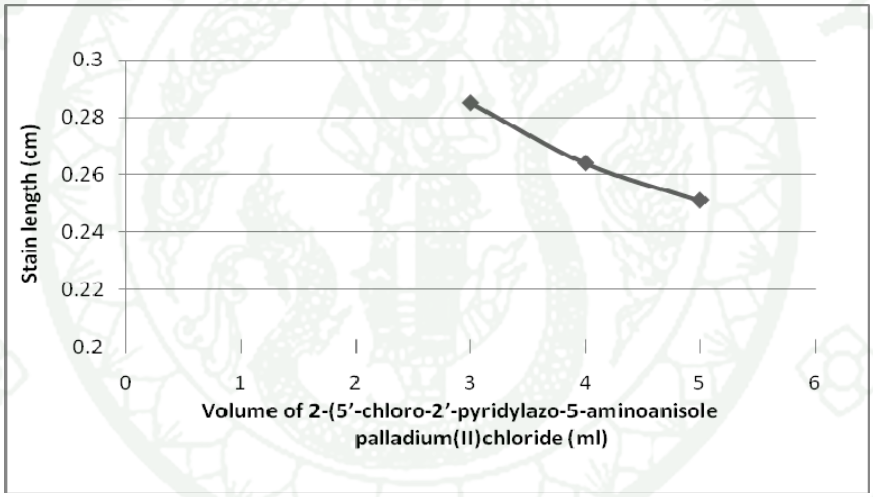


Figure 5 The effect of volume of 2-(5'-chloro-2'-pyridylazo-5-aminoanisole palladium(II)chloride coating on silica gel.

3.3. Optimization of amount of Zn powder on reduction of As(V) to As(III).

The of Zn powder was varied from 1.0 - 3.0g at 25 ppb As(V). The results are shown in Table 7 and Figure 6. The maximum stain length is obtained at 2.5 g of Zn and used for further study.

Table 7 Stain length of As(V) concentration at various amount of Zn powder.

Zn powder (g.)	Stain length (cm)
1.0	0.435
1.5	0.643
2.0	0.788
2.5	1.130
3.0	1.126

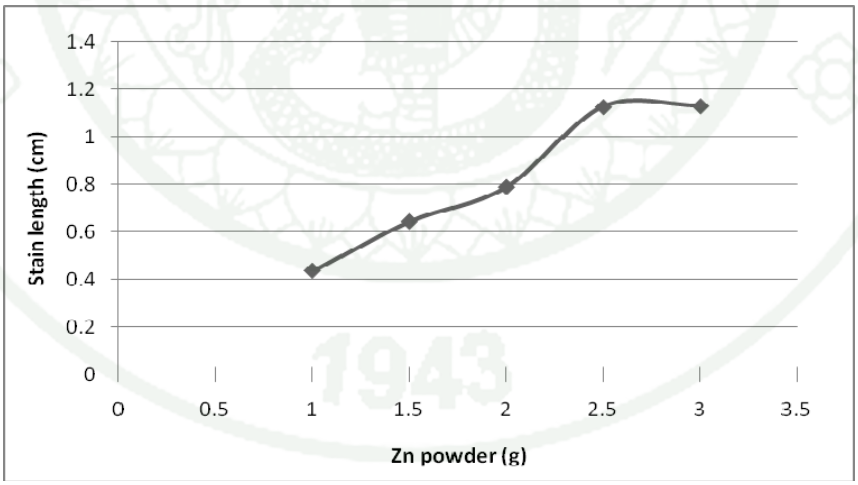


Figure 6 The effect of Zn powder on As(V) determination by detector tube.

3.4. Optimization amount of sulphamic acid.

The amount of sulphamic acid for As(V) determination at 25 ppb by detector tube method was studied in the range 2.0 – 3.5 g. As shown in Table 8 and Figure 7, the stain length of As(V) depends on the amount of sulphamic acid and after 2.5 g of sulphamic acid the stain length almost constant. The maximum stain length is obtained at 2.5 g. of sulphamic acid and used for further study.

Table 8 Stained length of As(V) concentration at various amount of sulphamic acid.

Sulphamic acid (g.)	Stain length (cm)
2.0	0.895
2.5	1.115
3.0	1.106
3.5	1.091

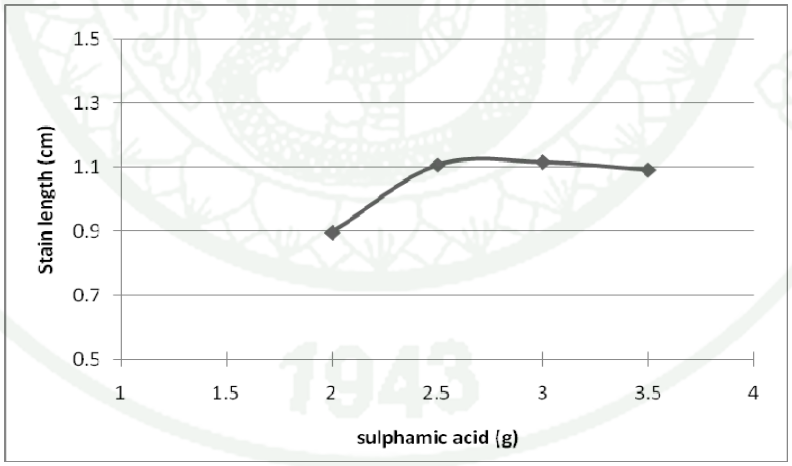


Figure 7 The effect of sulphamic acid on As(V) determination by detector tube.

3.5. Optimization of a detection time on stain length.

The detection time was varied in the range of 15 – 45 minute on As(V) determination. The results are shown in Table 9 and Figure 7. The maximum stain length is obtained at 35 minute As a results, A detection time at 35 minute is found to be suitable for 25 ppb As(V) determination by detector tube method.

Table 9 Stain length of As(V) concentration at various detection time.

Detection time (min)	Stain length (cm)
15	0.565
20	0.796
25	0.915
30	1.124
35	1.164
40	1.164
45	1.164

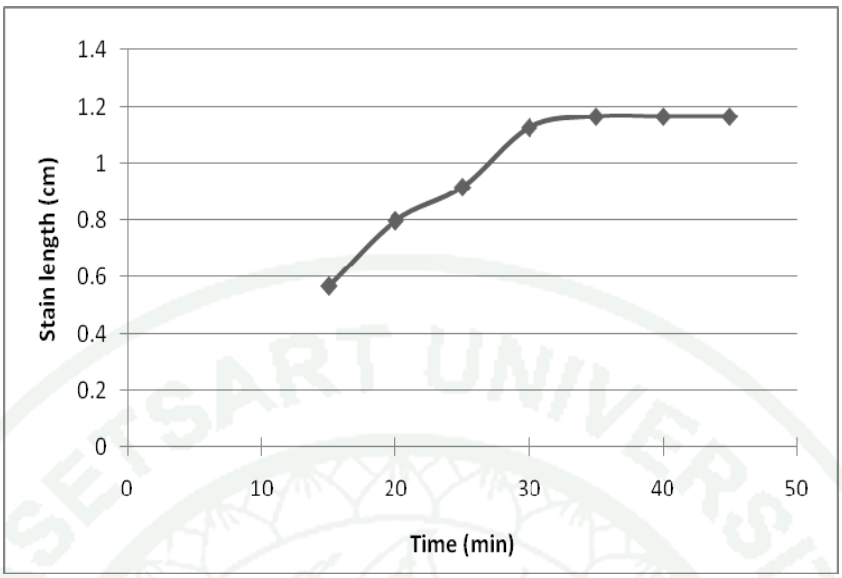


Figure 8 The effect of detection time on As(V) determination by detector tube.

3.6. Validation of the method.

3.6.1 Calibration curve.

Calibration curve of As(V) was linear from 5 ppb to 25 ppb with correlation coefficient of 0.9911. Calibration curve for As(V) was demonstrated in Figure 9. The sensitivity of the technique, measured in the term of the length of the stained layer per As(V) concentration obtained from calibration curve, was 0.3cm/5ppb.

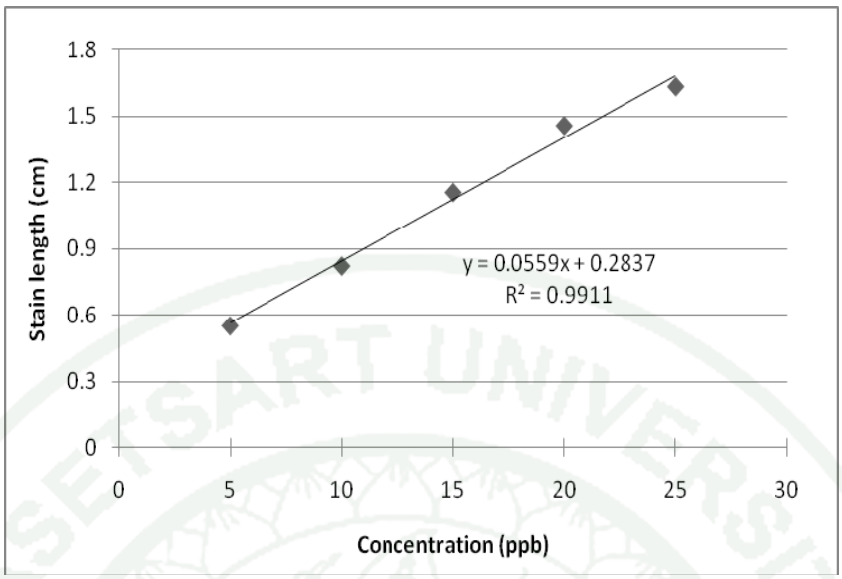


Figure 9 Calibration curve for As(V) / total arsenic.

3.6.2 Precision study.

The precision was studied at 5 and 20 ppb arsenic(V) with 10 replicates. The results are presented in Table 10 and the relative standard deviation (R.S.D.) was found to be 8.85% and 11.05% at 5 ppb and 20 ppb, respectively.

Table 10 The precision of As(V) using detector tube.

Replicate No.	Stain length of As(V) 5 ppb (cm)	Stain length of As(V) 20 ppb (cm)
1	0.569	1.429
2	0.509	1.595
3	0.485	1.684
4	0.575	1.334
5	0.569	1.415
6	0.455	1.340
7	0.569	1.303
8	0.575	1.347
9	0.533	1.216
10	0.611	1.201
Average color length (X)	0.545	1.386
Standard deviation (SD)	0.482	1.532
R.S.D%	8.85	11.05

R.S.D. = The relative standard deviation.

3.7 As(V) Interference study.

The effect of interfering ions was investigated at 10 ppb of As(V) concentration. Each ion was individually added to 10 ppb of As(V). The stain length of As(V) at 10 ppb was 0.532 ± 0.007 cm.

3.7.1 Effect of sulphide ion on As(V) detector tube. As shown in Table 11 the 250-fold of S(II) interfered positively.

Table 11 Effect of S(II) on determination of As(V).

S(II)/As(V) (mole ion ratio)	Stain length (cm)
0	0.530
150	0.556
200	0.546
250	0.836

3.7.2 Effect of tin(IV) ion on As(V) detector tube. As shown in Table 12 the 1000-fold of Sn(II) interfered positively.

Table 12 Effect of tin(IV) ion on the detection of As(V).

Sn(II) /As(V) (mole ion ratio)	Stain length (cm)
0	0.530
100	0.504
1000	0.665

3.7.3 Effect of bismuth(II) ion on As(V) detector tube. As shown in Table 13 the 30-fold of Bi(II) interfered positively.

Table 13 Effect of Bi(II) ion on the detection of As(V).

Bi(II) /As(III) (mole ion ratio)	Stain length (cm)
0	0.530
10	0.569
20	0.554
30	0.642

3.7.4 Effect of antimony(III) ion on As(V) detector tube. As shown in Table 14 the 250 molar fold of Sb(II) interfered positively.

Table 14 Effect of Sb(II) ion on determination of As(V).

Sb(II)/As(V) (mole ion ratio)	Stain length (cm)
0	0.530
50	0.519
150	0.521
250	0.609

3.7.5 Effect of As(III) ion on As(V) detector tube. As shown in Table 15 the 0.8 molar fold of As(III) interfered positively.

Table 15 Effect of As(III) ion on determination of As(V).

As(III) /As(V) (mole ion ratio)	Stain length (cm)
0	0.530
0.5	0.565
0.8	0.639
1	0.720

3.7.6 Effect of ferric(III) ion on As(V) detector tube. As shown in Table 16 the 50-fold of Fe(III) was interfered negatively.

Table 16 Effect of Fe(III) ion on determination of As(V).

Fe(III) /As(III) (mole ion ratio)	Stain length (cm)
0	0.530
10	0.503
50	0.422
100	0.460

3.7.7 Effect of mercury(II) ion on As(V) detector tube. As shown in Table 17 the 400-fold of Hg(II) interfered negatively.

Table 17 Effect of Hg(II) ion on determination of As(V).

Hg(II) /As(V) (mole ion ratio)	Stain length (cm)
0	0.530
300	0.508
400	0.326
500	0.254

3.7.8 Effect of disodium methylarsonate hexahydrate, MMAs(V) on As(V) detector tube. As shown in Table 18 the 3-fold of MMAs(V) interfered positively.

Table 18 Effect of MMAs(V) ion on determination of As(V).

MMAs(V)/As(V) (mole ion ratio)	Stain length (cm)
0	0.530
1	0.510
3	0.736
5	0.842

3.7.9 Effect of cacodylic acid, DMAs(V) on As(V) detector tube. As shown in Table 19 the 10-fold of DMAs(V) interfered positively.

Table 19 Effect of DMAs(V) ion on determination of As(V).

DMAs(V)/As(V) (mole ion ratio)	Stain length (cm)
0	0.530
1	0.552
5	0.594
10	0.636

From Table 11 – 19, it can be summarized that tolerance amount (mole ratio of ion : As(V)) of ions which interfere positively is 0.8, 1, 10 , 30, 55, 250 and 250 for As(III), MMAs(V), DMAs(V), Bi(II), S(II) and Sb(IV), respectively.

Tolerance amount (mole ratio of ion : As(V)) of ions which interfere negatively is 50 and 400 and 250 for Fe(III) and Hg(II), respectively.

3.8 Determination of well water samples using detector tube**.**

The method developed was used in the analysis of well water samples collected in Nakorn Si Thammarat as shown in Table 20.

Table 20 Determination of total arsenic in well water samples using a detector tube.

Sample Number	Concentration of total arsenic ($\mu g\ L^{-1}$)
1	4.07
2	5.38
3	2.77
4	9.05
5	44.00
6	21.32
7	not detectable
8	not detectable
9	not detectable
10	not detectable

4. Optimization of parameters on the determination of As(III) using a detector tube.

4.1 The effect of pH of 0.1M potassium hydrogen phthalate (KHP) buffer on As(III) determination.

The effect of pH on the arsine formation was examined pH of KHP was varied from pH 3 – pH 6. The results obtained are shown in Table 21 and the corresponding plot is illustrated in Figure 10. The maximum stain length was obtained at pH 4. This pH, thus, was selected for subsequent studies.

Table 21 Effect of various pH of 0.1M potassium hydrogen phthalate (KHP) buffer on As(III) determination.

pH of KHP buffer	Stain length (cm)
pH 3	1.590
pH 4	2.088
pH 5	2.084
pH 6	gas formation but no color change

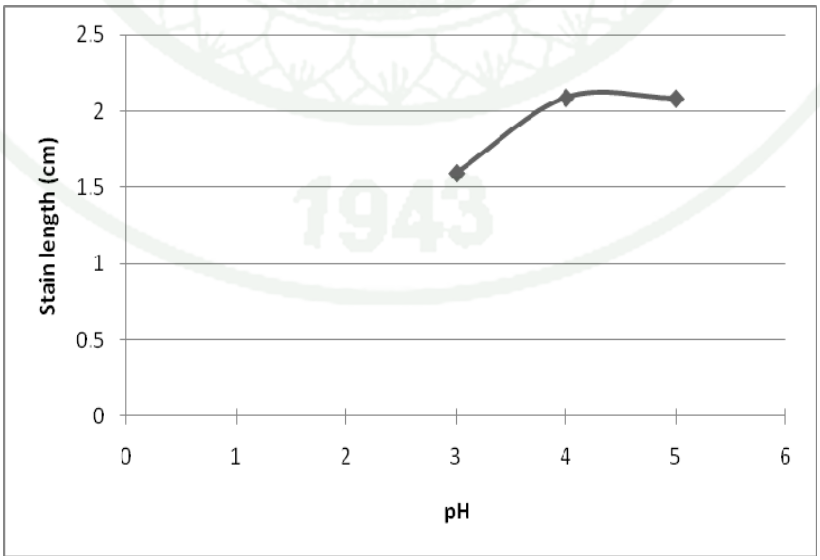


Figure 10 The effect of pH of 0.1M potassium hydrogen phthalate (KHP) buffer**.**

4.2 Optimization of concentration of $NaBH_4$ solution on As(III) determination.

The effect of varying concentration of $NaBH_4$ solution i.e 1%, 5%, 10%, 15% and 20% (%w/v) on determination of 25 ppb As(III) determination was investigated. The results are shown in Table 22 and Figure 11. The concentration of $NaBH_4$ solution 15% (%w/v) was used in the rest of the study.

Table 22 Effect of various concentration of $NaBH_4$ solution on As(III) determination.

Concentration of $NaBH_4$ (%w/v)	Stain length (cm)
1%	0
5%	1.113
10%	1.803
15%	2.088

20%	2.082

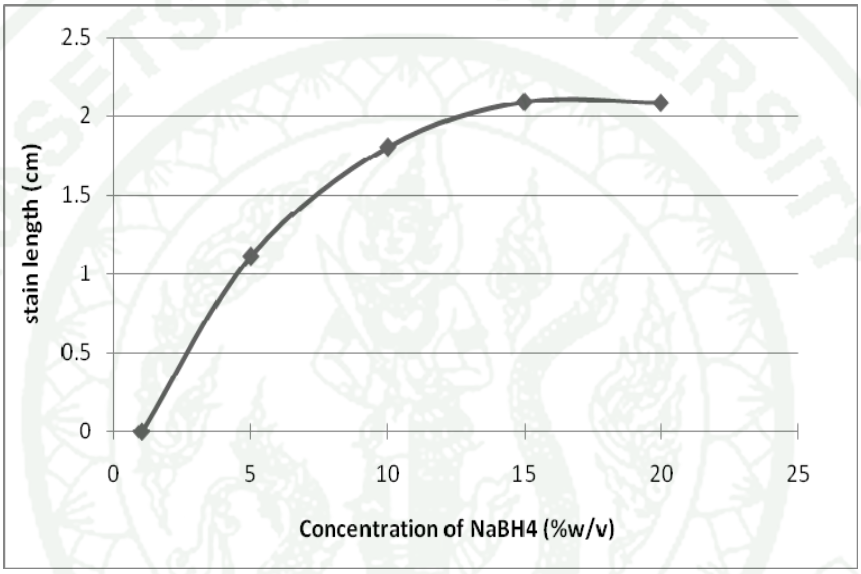


Figure 11 The effect of varying concentration of $NaBH_4$ solution on As(III) determination.

4.3 Optimization of detection time of a detector tube on As(III) determination.

The detection time was varies in the range of 5 - 20 minute on 25 ppb As(III) determination. The results are shown in Table 23 and Figure 11. The detection time 15 minute was used for As(III) determination with 0.1M KHP buffer pH 4 and 15% $NaBH_4$.

Table 23 Optimization of detection time on As(III) determination.

Detection time (min)	Stain length (cm)
5	1.994
10	2.099
12	2.119
15	2.200
18	2.200
20	2.200

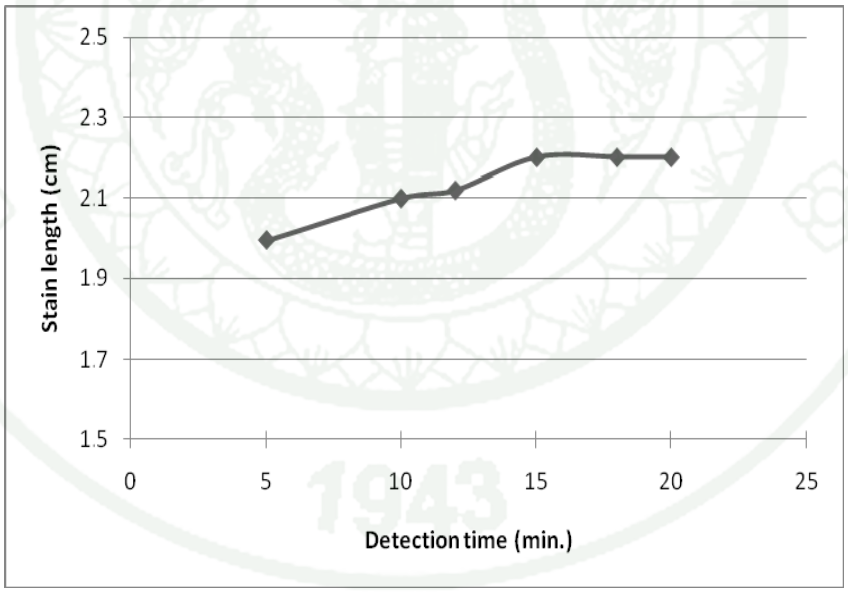


Figure 12 The effect of varying detection time on As(III) determination.

4.4. Validation of the method.

The As(III) standard solution were user for validation of the method in this work.

4.4.1 Calibration curve.

Calibration curve of As(III) was linear from 5 ppb to 25 ppb with correlation coefficient of 0.9913. Calibration curve for As(III) was demonstrated in Figure 13. The sensitivity of the technique, measured in the term of the length of the stained layer per As(III) concentration obtained from calibration curve, was 0.53cm/5ppb.

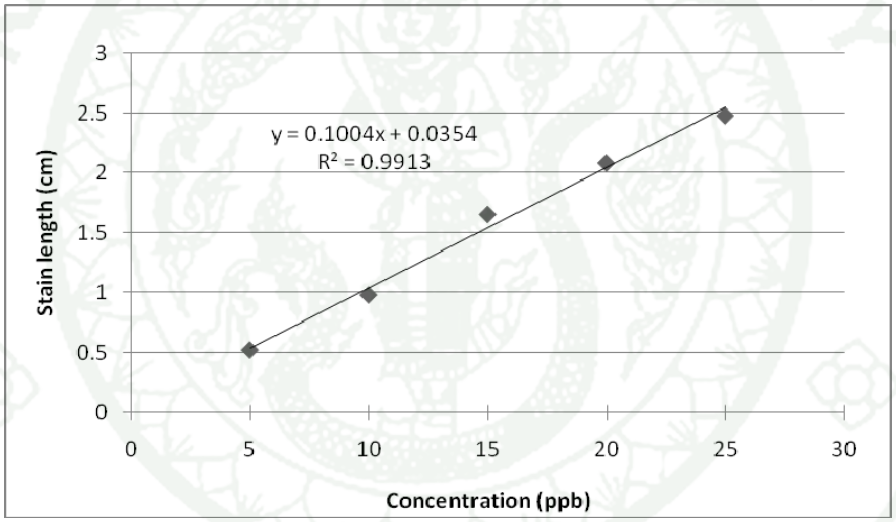


Figure 13 Calibration curve for As(III) by detector tube method.

4.4.2 Precision.

The precision was studied at 10 ppb As(III) and measurement 6 replicates, respectively. The results were presented in the Table 24 and the relative standard deviation (R.S.D.) was calculated to be 13.07% at 10 ppb, respectively.

Table 24 The precision of As(III) using detector tube.

Replicate No.	Stain length of As(III) 10 ppb (cm)
1	0.773
2	0.707
3	0.681
4	0.918
5	0.928
6	0.850
Average color length (X)	0.809
Standard deviation (SD)	1.058
R.S.D%	13.07

R.S.D. = The relative standard deviation

4.5 As(III) Interference study.

The effect of interfering ions was investigated at 10 ppb of As(V) concentration. Each ion was individually added to 10 ppb of As(V). The stain length of As(V) at 10 ppb was 0.861 ± 0.012 cm.

4.5.1 Effect of sulphide ion on As(III) detector tube. As shown in Table 25 the 40-fold of S(II) interfered positively.

Table 25 Effect of S(II) ion on detection of As(III).

S(II) /As(III) (mole ion ratio)	Stain length (cm)
0	0.825
10	0.824
30	0.812
40	1.192

4.5.2 Effect of tin(IV) ion on As(III) detector tube. As shown in Table 26 the 100- fold of Sn(IV) ion interfered positively.

Table 26 Effect of Sn(IV) ion on detection of As(III).

Sn(IV)/As(III) (mole ion ratio)	Stain length (cm)
0	0.825
70	0.827
90	0.843
100	0.902

4.5.3 Effect of bismuth(II) ion on As(III) detector tube. As shown in Table 27 the 9- fold of Bi(II) interfered positively.

Table 27 Effect of bismuth(II) ion on detection of As(III).

BI(II)/As(III) (mole ion ratio)	Stain length (cm)
0	0.825
7	0.866
9	0.968
10	1.183

4.5.4 Effect of Antimony(III) ion on As(III) detector tube. As shown in Table 28 the 20-fold of Sb(II) interfered positively.

Table 28 Effect of Sb(II) ion on determination of As(III) .

Sb(II) /As(III) (mole ion ratio)	Stain length (cm)
0	0.825
10	0.816
20	1.012
30	1.061

4.5.5 Effect of As(V) ion on As(III) detector tube. As shown in Table 29 the 10-fold of As(V) interfered positively.

Table 29 Effect of As(V) ion on determination of As(III).

As(V)/As(III) (mole ion ratio)	Stain length (cm)
0	0.825
5	0.824
7	0.813
10	1.008

4.5.6 Effect of Ferrous(III) ion on As(III) detector tube. As shown in Table 30 the 10-fold of Fe(III) over As interfered negatively.

Table 30 Effect of Fe(III) ion on determination of As(III).

Fe(III) /As(III) (mole ion ratio)	Stain length (cm)
0	0.825
10	0.480
50	0.501
100	0.560

4.5.7 Effect of mercury(II) ion on As(III) detector tube. As shown in Table 31 the 100-fold of Hg(II) interfered positively.

Table 31 Effect of Hg(II) on determination of As(III).

Hg(II) /As(III) (mole ion ratio)	Stain length (cm)
0	0.825
10	0.831
50	0.829
100	0.930

4.5.8 Effect of disodium methylarsonate hexahydrate, MMAs(V) on As(III) detector tube. As shown in Table 32 the 30-fold of MMAs(V) interfered positively.

Table 32 Effect of MMAs(V) ion on determination of As(III).

MMAs(V)/As(III) (mole ion ratio)	Stain length (cm)
0	0.825
20	0.835
30	0.943
50	1.360

4.5.9 Effect of cacodylic acid, DMAs(V) on As(III) detector tube. As shown in Table 33 the 4-fold of DMAs(V) interfered positively.

Table 33 Effect of DMAs(V) ion on determination of As(III).

DMAs(V)/As(III) (mole ion ratio)	Stain length (cm)
0	0.825
3	0.877
4	0.932
5	1.318

From Table 25 – 33, it can be summarized that tolerance amount (mole ratio of ion : As(III)) of ions which interfere positively is 4, 9, 10 , 20, 30, 40, 100 and 100 molar fold for DMAs(V), Bi(II), As(V), MMAs(V), S(II), Hg(II) and Sn(IV), respectively.

Tolerance amount (mole ratio of ion: As(III)) of ions which interfere negatively is 10 molar fold for Fe(III), respectively.

4.6 Determination of well water samples using detector tube**.**

The method developed was used in the analysis of well water samples collected in Nakorn si Thammarat as shown in Table 34.

Table 34 Determination of As(III) in well water samples using detector tube.

Sample Number	Concentration of As(III) (μg L^{-1})
1	not detectable
2	7.56
3	not detectable
4	not detectable
5	9.05
6	not detectable
7	not detectable
8	not detectable
9	not detectable
10	not detectable

5. Optimization parameters on the reduction of As(V) to As(III).

From preliminary experiments, we found that sodiumborohydride method gave higher sensitivity for As(III) than that of Zn method. Therefore, pre-reducing agent (L-cysteine, thiourea, potassium iodide, ascorbic acid and hydroxylamine hydrochloride) were tried to convert As(V) to As(III) for determination of As(V) as Figure 4. The methods were carried out as described in section 5.1 – 5.5. The results are shown in Table 35.

Table 35 Screening of pre-reductants for conversion of As(V) to As(III).

Pre-reducing agent	Stain length (cm)
L-cysteine	Colour change from blue to brown color by blank determination
Thiourea	Colour change from blue to yellow color by blank determination
Potassium iodide	Gas formation but no color change
Ascorbic acid	Gas formation but no color change
Hydroxy ammonium chloride	Gas formation but no color change

As shown in Table 35, there was no appropriate prereductant found for conversion of As(V) to As(III). This approach was abandoned.

Part 2 : Hydride generation atomic absorption technique method.

1. Two acid media were chosen comparative study on As(III) determination by hydride generation atomic absorption spectrometry.

A solution mixture containing 15 ppb As(III) and 75ppb As(V) was used throughout the comparison study. Percentage difference between As(III) in pure solution and in a mixture was calculated by comparing the absorbance obtained from the mixture with that obtained from pure solution.

1.1 Tris-(hydroxymethyl)aminomethane hydrochloride as a selective medium for As(III) determination by HG-AAS.

1.1.1 Effect of pH of 2.5M tris-(hydroxymethyl)aminomethane hydrochloride was varied from 5 - 9 at 2% $NaBH_4$ (%w/v) in 0.02M NaOH. The results are shown in Figure 14 and figure 15.

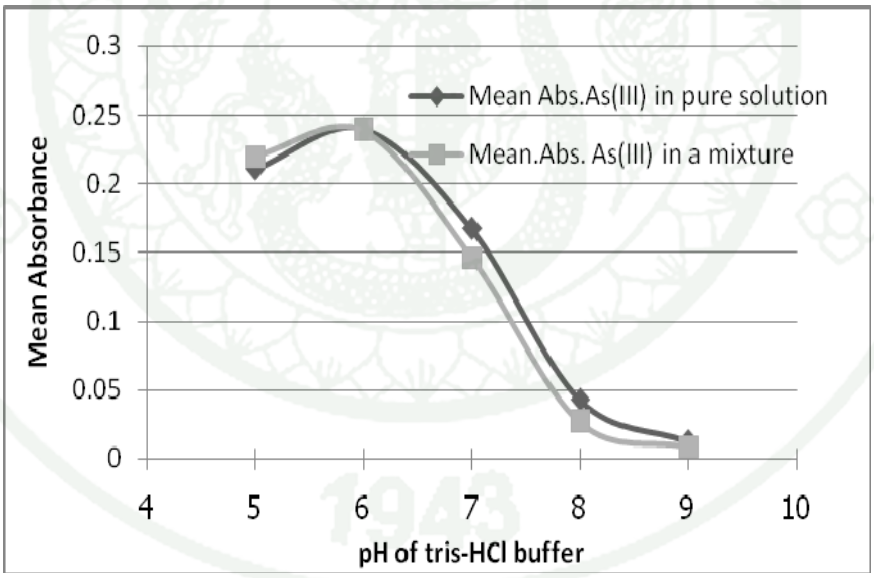


Figure 14 Effect of pH of tris-HCl buffer at 2% $NaBH_4$ (%w/v) in 0.02M NaOH on mean absorbance.

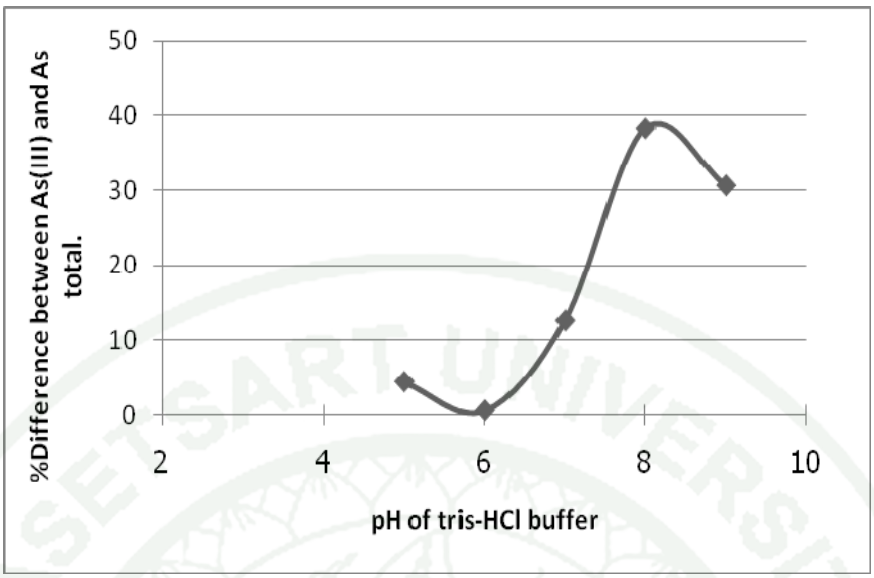


Figure 15 Effect of pH of tris-HCl buffer at 2% $NaBH_4$ (%w/v) in 0.02M NaOH on % difference between As(III) in pure solution and in a mixture.

As shown in Figure 14 maximum mean absorbance of 15 ppb As(III) was 0.2400 and in Figure 15 the minimum % difference between As(III) in pure solution and in a mixture was achieved at pH 6 at 2% $NaBH_4$ (%w/v) in 0.02M NaOH. For the subsequent experiments, pH 6 of 2.5M tris-HCl buffer was selected for As(III) analysis.

1.1.2 Effect of sodium tetrahydroborate concentration was studied by varying its concentration from 0.5% to 4% at tris-HCl (pH 6). The result was shown in Figure 16 and Figure 17.

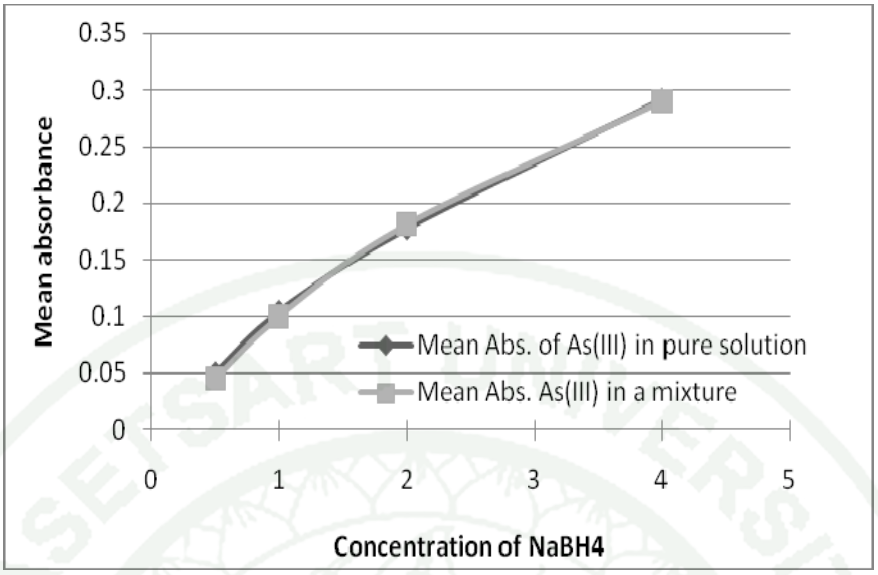


Figure 16 Effect of sodium tetrahydroborate at pH6 2.5M tris-HCL buffer on mean absorbance.

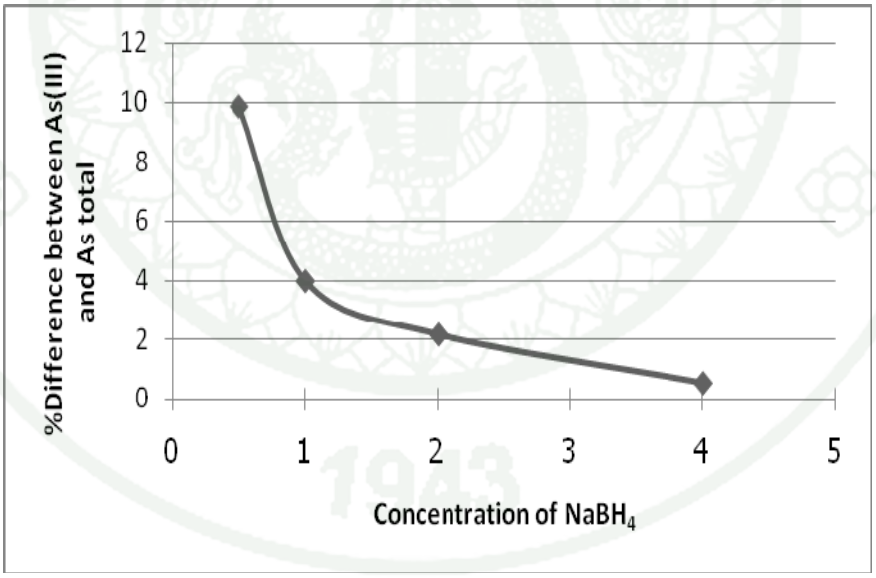


Figure 17 Effect of sodium tetrahydroborate at pH6 2.5M tris-HCL buffer on % difference between As(III) in pure solution and in a mixture.

As shown in Figure 16 shows a maximum mean absorbance of 15 ppb As(III) was 0.2981 and Figure 17 indicates that minimum % difference between As(III) in a pure solution and in a mixture was obtained at 4% $NaBH_4$(w/v) in 0.02M NaOH was used in subsequent experiments.

1.1.3 Effect of sodium hydroxide concentration was varied from 0.02 - 0.2M at 2.5M tris-HCl pH 6. The results are shown in Figure 18 and Figure 19.

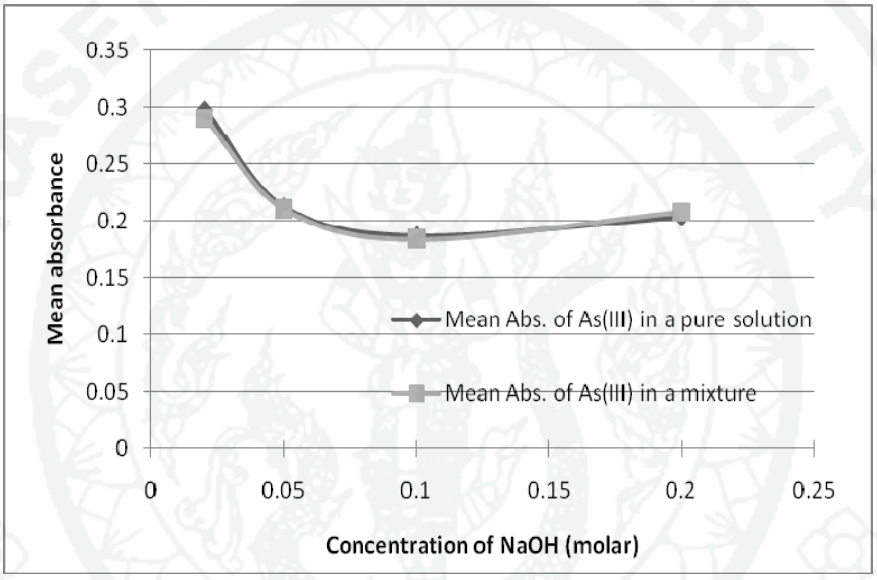


Figure 18 Effect of sodium hydroxide at pH6 2.5M tris-HCl buffer on mean absorbance.

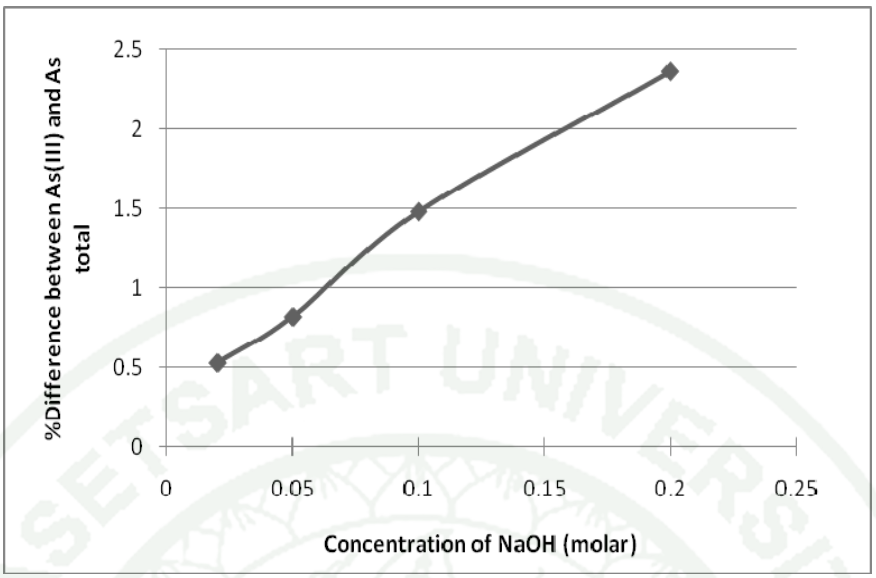


Figure 19 Effect of sodium hydroxide at pH6 2.5M tris-HCl buffer on % difference between As(III) in a pure solution and in a mixture.

As shown in Figure 18 shows a maximum mean absorbance of 15 ppb As(III) was 0.2918 and Figure 19 indicated that % difference between As(III) in a pure solution and in a mixture of 0.52% was minimum with relative standard deviation of 1.04% at 2.5M tris-HCl pH6 with 4% $NaBH_4$(w/v) in 0.02M NaOH.

1.2 Citric acid as a selective medium for As(III) determination by HG-AAS.

1.2.1 The concentration of citric acid was varied from 0.05-1.50 M at 0.5% $NaBH_4$ (w/v) in 0.5% NaOH. The results are shown in Figure 20 and Figure 21.

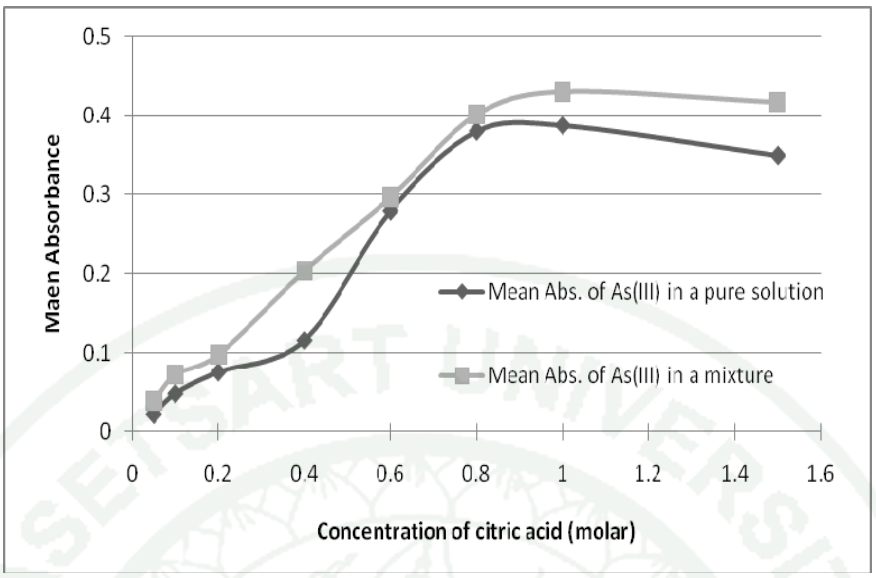


Figure 20 Effect of citric acid concentration on mean absorbance of As(III) in a pure solution and in a mixture.

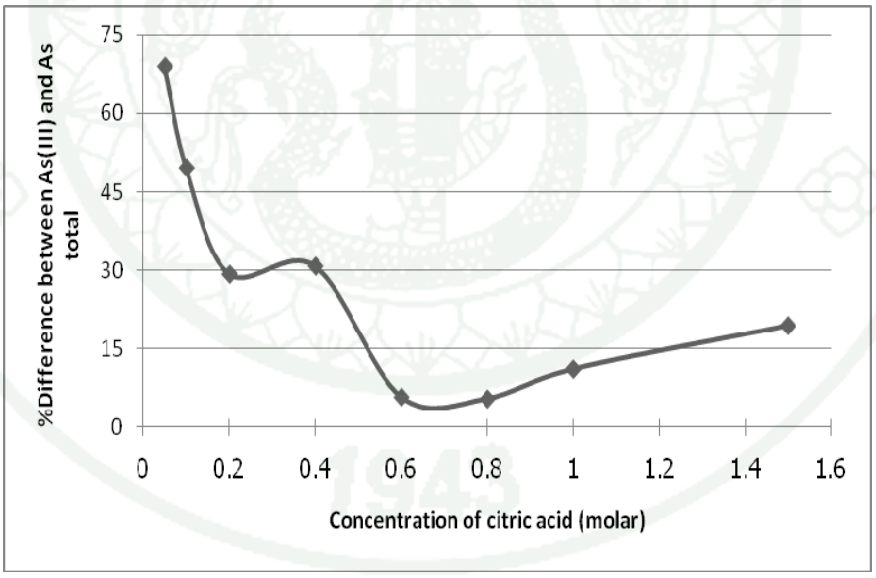


Figure 21 Effect of citric acid concentration on % difference between As(III) in a pure solution and in a mixture.

Citric acid at 1.0M was chosen. At this concentration gave a maximum absorbance of 15 ppb As(III) 0.3874 and % difference between As(III) in pure solution and in a mixture of 11.20% were obtained.

1.2.2 Effect of sodium borohydride concentration was varied from 0.5% - 3.5% in 1.0M citric acid. The result is shown in Figure 22 and Figure 23.

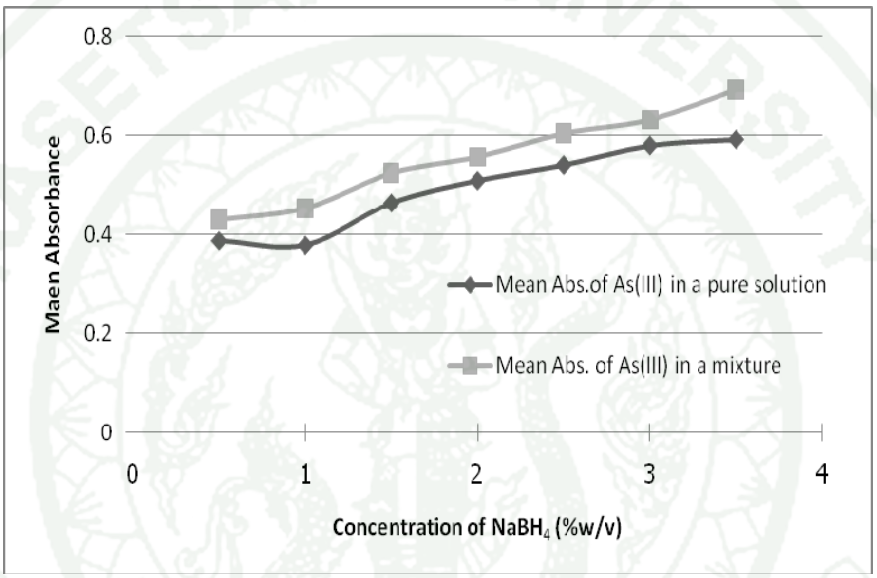


Figure 22 Effect of sodium borohydride in 1.0M citric acid on mean absorbance of As(III) in a pure solution and in a mixture.

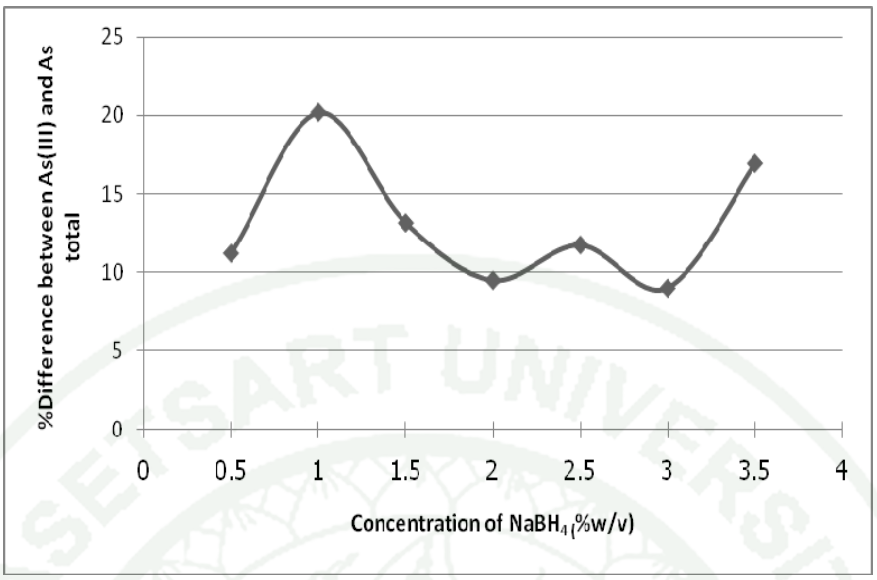


Figure 23 Effect of sodium borohydride in 1.0M citric acid on % difference between As(III) in a pure solution and in a mixture.

As shown in Figure 22 shows a maximum mean absorbance of 15 ppb As(III) was 0.2918 and Figure 23 indicated that % difference between As(III) in a pure solution and in a mixture 11.18% were obtained.

1.2.3 Effect of sodium hydroxide concentration was varied from 0.5% - 1.0% in 1.0M citric acid. The result is shown in Figure 24 and Figure 25.

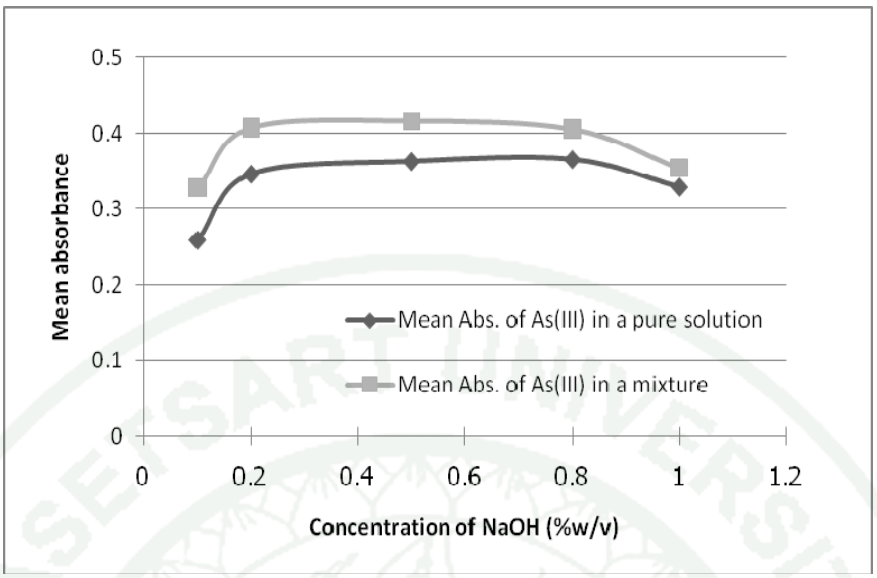


Figure 24 Effect of sodium hydroxide in 1.0M citric acid on mean absorbance of As(III) in a pure solution and in a mixture.

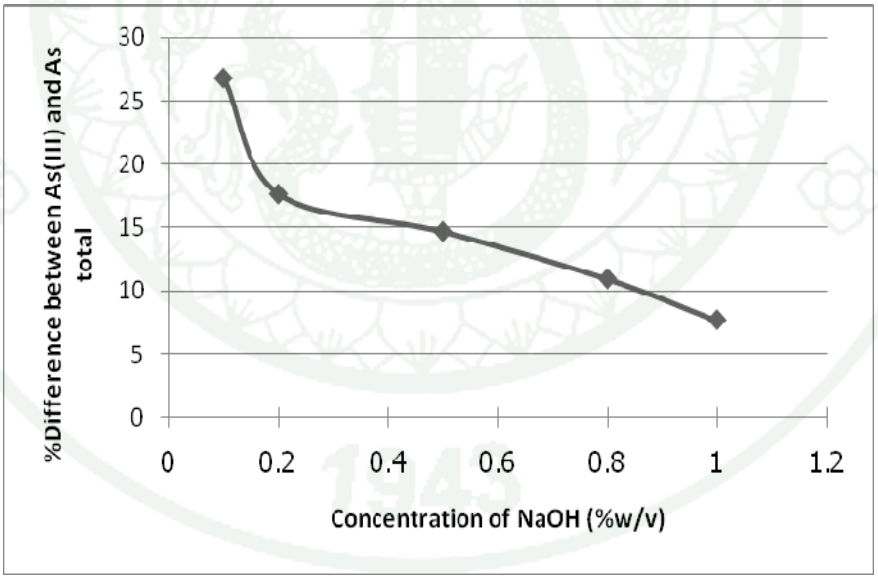


Figure 25 Effect of sodium hydroxide in 1.0M citric acid on % difference between As(III) in a pure solution and in a mixture.

As shown in Figure 24 shows a maximum mean absorbance of 15 ppb As(III) was 0.2918 and Figure 25 indicated that % difference between As(III) in a pure solution and in a mixture of 14.69% were obtained.

When compared to tris-HCl buffer medium, citric acid medium gives more precision (less relative standard deviation) with higher absorbance of As(III) and used for further work. A method for determination of inorganic arsenic speciation was developed by using selective acid medium in the hydride generation of arsine from As(III) and total arsenic and As(V) followed the standard protocol of hydride generation atomic absorption spectrometry.

2. Validation method

2.1 Calibration curve for As(III) by hydride generation atomic absorption spectrometry.

The operating condition of the hydride generation atomic absorption system for As(III) as shown in Table 3 and In citric acid medium, linear calibration curve for As(III) was obtained in the range 1 ppb to 20 ppb with R^2 of 0.9931 as shown in Figure 26.

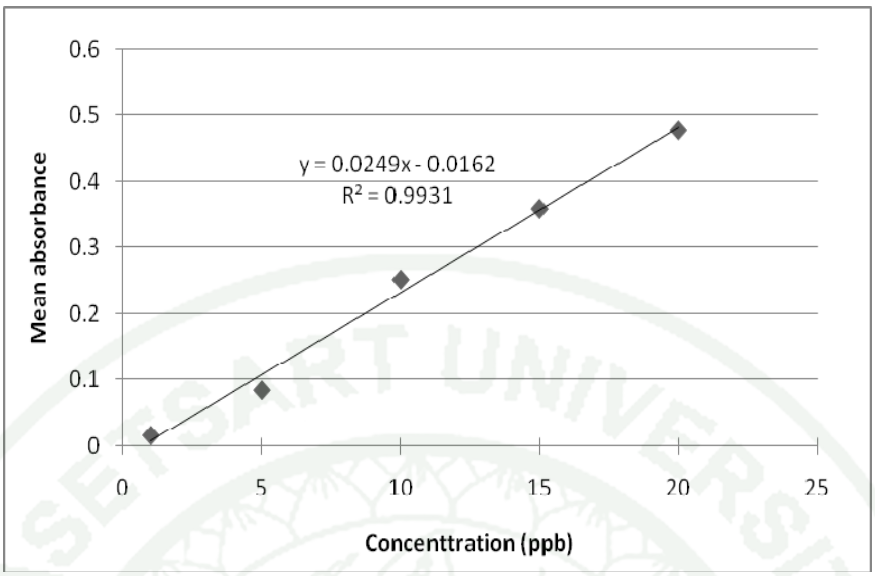


Figure 26 A plot of As(III) concentration against absorbance.

2.2 Calibration curve for As total determination by hydride generation atomic absorption spectrometry.

The operating condition of the hydride generation atomic absorption system for As(V) as shown in Table 4 and In citric acid medium, linear calibration curve for As(V) was obtained in the range 1 ppb to20 ppb with R^2 of 0.9972 as shown in Figure 27.

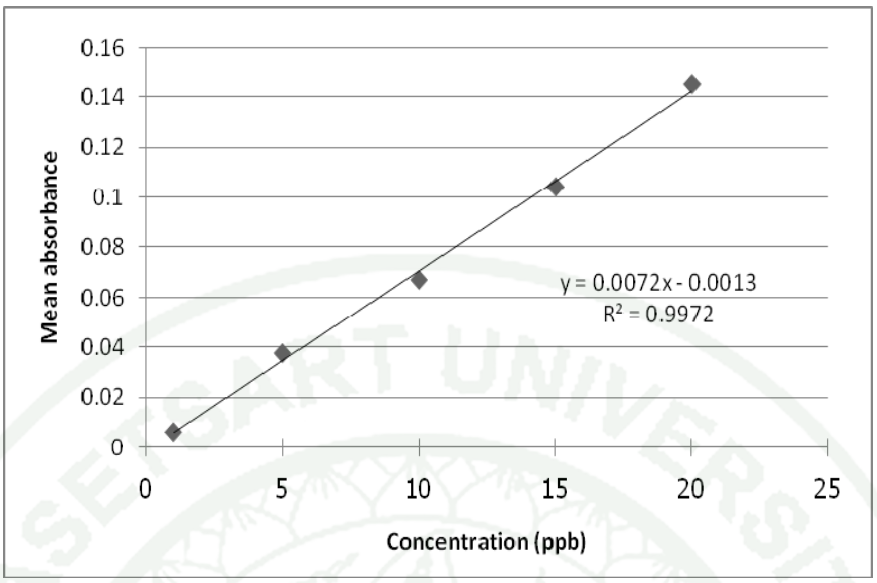


Figure 27 A plot of total arsenic concentration against absorbance.

2.3 Determination of well water samples using hydride generation atomic absorption spectrometry.

The method developed was used in the analysis of well water samples collected in Nakorn Si Thammarat as shown in Table 3 and Table 4. Of ten sample, four sample contained total arsenic content higher than 10 ppb. The results are shown in Table 36.

Table 36 Determination of As(III) and total arsenic in well water samples using hydride generation atomic absorption spectrometry.

Sample Number	As(III) μg L^{-1}	Total As μg L^{-1}
1	3.080	5.036
2	6.820	25.379
3	0.517	3.560
4	4.144	13.250
5	6.528	50.810
6	4.204	17.797
7	2.160	0.554
8	1.832	0.405
9	1.960	0.506
10	1.828	0.405

The inorganic arsenic speciation can be performed by using citric acid as a selective medium for As(III) determination. In this medium, the precision for As(III) is better than that in tris-HCl medium. Total arsenic and As(V) can be determined in conventional KI and HCl medium. Of ten samples, four samples contained total arsenic higher than 10 ppb.

2.4 Precision study.

The precision of determination of As(III) was examined at 5 ppb, 10 ppb and 20 ppb arsenic(III) with 10 replicates. The results were presented in Table 37

and the relative standard deviation (R.S.D.) was calculated to be 5.38%, 1.96% and 3.57% at 5 ppb, 10 ppb and 20 ppb, respectively.

The precision of determination of total arsenic was examined at 5 ppb, 10 ppb and 20 ppb arsenic(V) with 10 replicates. The results were presented in Table 38 and the relative standard deviation (R.S.D.) was calculated to be 3.05%, 1.23% and 1.24% at 5 ppb and 10 ppb and 20 ppb, respectively.

Table 37 The precision of As(III) using hydride generation atomic absorption spectrometry method.

Replicate No.	Absorbance of As(III) 5 ppb	Absorbance of As(III) 10 ppb	Absorbance of As(III) 20 ppb
1	0.1424	0.3110	0.5047
2	0.1550	0.2999	0.5090
3	0.1567	0.3023	0.5218
4	0.1680	0.3122	0.4755
5	0.1654	0.3080	0.4841
6	0.1648	0.3167	0.4909
7	0.1663	0.3123	0.5069
8	0.1705	0.3128	0.5187
9	0.1683	0.3198	0.5341
10	0.1688	0.3145	0.4958
Average absorbance(X)	0.1626	0.3108	0.5041
Standard deviation (SD)	0.008	0.006	0.018
RSD %	5.38	1.96	3.57

Table 38 The precision of total arsenic using hydride generation atomic absorption spectrometry method.

Replicate No.	Absorbance of total As 5 ppb	Absorbance of total 10 ppb	Absorbance of total 20 ppb
1	0.0456	0.0647	0.1881
2	0.0433	0.0647	0.1900
3	0.0435	0.0647	0.1889
4	0.0418	0.0646	0.1849
5	0.0437	0.0648	0.1833
6	0.0442	0.0648	0.1871
7	0.0422	0.0648	0.1872
8	0.0430	0.0648	0.1843
9	0.0428	0.0649	0.1856
10	0.0459	0.0648	0.1835
Average absorbance(*X*)	0.0436	0.3108	0.1862
Standard deviation (SD)	0.0013	0.006	0.002
RSD%	3.05	1.23	1.24

3. Comparison of an analysis of As(III), As(V) and total arsenic in well water samples using the present method versus HG-AAS.

Accuracy of the detector tube was also evaluated by comparison with the instrumental method. The results are shown in Table 39 and Table 40.

Table 39 Determination of As(III) in well water samples using a detector tube compared with HG-AAS method.

Water Sample	Concentration of As(III) ($\mu g\ L^{-1}$)	
	Detector tube method	HG-AAS method
1	not detectable	3.08
2	7.56	6.82
3	not detectable	4.14
4	not detectable	0.36
5	9.05	6.52
6	not detectable	4.20
7	not detectable	2.16
8	not detectable	1.83
9	not detectable	1.96
10	not detectable	1.82

Table 40 Determination of As(V) and total arsenic in well water samples using a detector tube compared with HG-AAS method.

Water Sample	Detector tube		HG-AAS	
	As (V) µg L^{-1}	Total As µg L^{-1}	As (V) µg L^{-1}	Total As µg L^{-1}
1	4.07	4.07	1.943	5.06
2	5.38	12.94	6.820	25.37
3	2.77	2.77	0.517	3.56
4	9.05	9.05	4.144	13.25
5	44.00	53.05	6.528	50.81
6	21.32	21.23	4.204	17.79
7	not detectable	not detectable	2.160	0.544
8	not detectable	not detectable	1.832	0.405
9	not detectable	not detectable	1.960	0.506
10	not detectable	not detectable	1.828	0.405

As shown in Table 39 and Table 40, the results obtained from both methods agree well except sample No.2 which the present method gave a lower value. This may be due to the interfering ions present in the water sample.

CONCLUSION

A new detector tube for arsenic analysis in well water samples was developed. The method based on conversion of arsenic in water to arsine. The generated arsine was passed into the detector tube containing a new color complex of 2-(5′-chloro-2′-pyridylazo)-5-aminoanisolepalladium(II) chloride as a sensing materials coated on silica gel the color of the sensing materials changed from purple to yellow and the length of color change is proportional to concentration of arsine. Total inorganic As(III) and As(V) was changed to arsine using Zn and sulphamic acid while arsenic(III) was changed to arsine using $NaBH_4$. The linear range of 5 – 25 ppb for both As(III) and total inorganic arsenic with coefficient of determination of 0.9913 and 0.9911, respectively, were obtained. The precision of arsenic determination at 10 ppb (n=6) was 13.07% for As(III), and at 5 ppb and 20 ppb (n=10) were 8.85%, 11.05% for total arsenic.

The method for inorganic arsenic speciation using hydride generation atomic absorption spectrometry was also developed. Therefor, determination of As(III) was performed in citric acid. Total arsenic was determined in conventional KI and HCl medium. The detection limit for As(III) was 0.02 $\mu g/L^{-1}$ and that for total arsenic was 0.23 $\mu g/L^{-1}$. The linear rang of 1 – 20 ppb for As(III) was 0.9931 and As(V) was 0.9972. The precision of arsenic determination at 5 ppb, 10 ppb and 20 ppb (n=10) were 5.38% 1.96%, 3.57 for As(III), and 3.05%, 1.23%, 1.24 for total arsenic. Of ten samples from Ronpiboon distrinct Nakorn Si Thaammarat, four samples contained total arsenic higher than 10 ppb.

LITERATURE CITED

_____ . 2011. Thailand Map (Online) http://www.whereig.com/thailand/map-political.html

Abdel-Lateef, A.M., Mohamed, R.A., and Mahmoud, H.H. 2013. Determination of Arsenic (III) and (V) Species in Some Environmental Samples by Atomic Absorption Spectrometry. **Advances in Chemical Science.** 2: 110-113.

Akter, K., Chena, Z., Smith, L., Davey, D., Naidu, R. 2005. Speciation of arsenic in ground water samples: A comparative study of CE-UV, HG-AAS and LC-ICP-MS. **Talanta.** 68: 406–415.

Almela, C., Clemente, M., Ve´lez, D., Montoro, R. 2006. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. **Food and Chemical Toxicology.** 44: 1901–1908.

Anawara, H. 2012. Arsenic speciation in environmental samples by hydride generation and electrothermal atomic absorption spectrometry. **Talanta.** 88: 30 - 42.

Burguera, M., and Burguera, J., 1997. Analytical methodology for speciation of arsenic in environmental and biological samples. **Talanta.** 44(9): 1581-1604.

Chakma, S., Rahman, M., Islam, P., and Haq, M., 2012. Arsenic in rice and rice straw. **The Bangladesh Veterinarian.** 29:1-6.

Chanthai, S., Suwamat, N., Ruangviriyachai, C. and Danvirutai, P. 2007. Speciation analysis of arsenic (III), arsenic (V) and total arsenic by continuous flow HG-

AAS in Thai Fruit Wines and Distilled Spirits. **ASEAN Food journal.** 14: 181-196.

Cherukuri, J., and Anjaneyulu. 2005. Design and Development of Low Cost, Simple, Rapid and Safe, Modified Field Kits for the Visual Detection and Determination of Arsenic in Drinking Water Samples. **Int. J. Environ. Res. Public Health.** 2(2): 322–327.

Delgado-Andrade, C., Navarro, M., Lo´pez, H., and Lo´pez, M.C. 2003. Determination of total arsenic levels by hydride generation atomic absorption spectrometry in foods from south-east Spain: estimation of daily dietary intake. **Food Additives and Contaminants.** 20: 923–932.

Doker, S., Uzun, L., Denizli, A., 2013. Arsenic speciation in water and snow samples by adsorption on to PHEMA in a micro-pipette-tip and GFAAS detection applying large-volume injection**. Talanta.** 103: 123–129.

Erdoğan, H., Yalçınkaya, O., Türker, A. 2011. Determination of inorganic arsenic species by hydride generation atomic absorption spectrometry in water samples after pre-concentration/separation on nano ZrO_2/B_2O_3 by solid phase extraction. **Desalination.** 280: 391–396.

Gong, Z., Lu, X., Ma, M., Watt, C., and Le, X. 2002. Arsenic speciation analysis. **Talanta**. 58: 77-96.

Gonzalez, J., Lavilla, I., and Bendicho, C. 2003. Evlution of non-chromatographic approaches for speciation of extractable As(III) and As(V) in environmental solid samples by FI-HG-AAS. **Talanta**. 59: 525-534.

HarkabuSoVá, V., MaCHaráčkoVáv, B., čeleCHoVSk, O., and VItouloVá, E. 2009. Determination of Arsenic in the Rainbow Trout Muscle and Rice Samples. **Czech J. Food Sci.** 27: 404-406

Heitland, P., and Kisster, H. 2008. Fast Determination of Arsenic Species and Total Arsenic in Urine by HPLC-ICP-MS: Concentration Ranges for Unexposed German Inhabitants and Clinical Case Studies. **Journal of Analytical Toxicology**. 32: 308-314.

Hu, S., Lu, J., Jing, C. 2012. A novel colorimetric method for field arsenic speciation analysis**. Journal of Environmental Sciences.** 24(7): 1341–1346.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2004. Some Drinking-water Disinfectants and Contaminants, including Arsenic. ISBN 92 832 1284 3.

Jain, C.K. and Ali, I. 2000. Arsenic: occurrence, toxicity and speciation technique. **Wat. Res**. 34: 4304-4312.

Janati, S., Beheshti, H., and Fahim, N. 2011. Determinations of Cadmium, Lead, Arsenic and Mercury in rice from Iran. **International journal of Industrial chemistry.**

Jeremiah, O., Ruth, W., Jane, M. and Charles, O. 2013. Analysis of the levels of arsenic in home-made brews, spirits, in water and raw materials using Hgaas in Nairobi county. **African Journal of Pure and Applied Chemistry**. 7: 291-301.

Karak, T., Abollinob, O., Bhattacharyyac, P., Dasd, K. and Paule R. 2011. Fractionation and speciation of arsenic in three tea gardens soil profiles and distribution of As in different parts of tea plant (Camellia sinensisL.). **Chemosphere.** 85: 948–960

Khim, O., Ismail, N., Jamal, S., Jabit, M. and Azmi, N. 2010. Development of arsenic detection test kit for military field drinking water. **The Journal of Defence and Security.** 1: 251 – 257.

Koesmawati, T., Buchari, B., Amran, M., Kardono, L. 2013. Determination of Total Arsenic in Indonesian Tuna Fish Sample. **Journal of Applied Pharmaceutical Science.** 3: 116-121

Korenovska, M., and Suhaj, M. 2005. Application of GF-AAS Methods for As^{3+} and As^{5+} Determination in Fish Products. **Chem.Pap.** 59: 153-156.

Maity, S., Chakravarty, S., Thakur, P. and Roy B. 2004. Evalution and standardization of a simple HG-AAS method for rapid speciation of As(III) and As(V) in some contaminated groundwater samples of West Bengal, India. **Chemosphere.** 54: 1199-1206.

Musil, S. and Matoušek T. 2008. On-line pre-reduction of pentavalent arsenicals by thioglycolic acid for speciation analysis by selective hydride generation–cryotrapping–atomic absorption spectrometry. **Spectrochimica Acta.** Part B 63: 685–691.

Niedzielski, P. and Siepak, M. 2003. Analytical Methods for Determining Arsenic, Antimony and Selenium in Environmental Samples. **Polish Journal of Environmental Studies**. 12: 653-667.

Pan, Q., Liu, W., Yang, J., Min, Y., and Huang, Z. 2013. Determination of Arsenic in *Panax notoginseng* by HydrideGeneration Atomic Fluorescence pectrometry. **Asian Journal of Chemistry.** 25(6):2959-2961.

Rahman, Md.m., Fujinaga, K., Seike, Y., and Okumura, M. 2004. A Simple in situ Visual and Tristimulus Colorimetric Method for the Determination of Trace Arsenic in Environmental Water rafter Its Collection on a Mercury(II)-Impregnated Paper**. Analytical Science.** 20: 165-170.

Rasmussen, R., Hedegaard, R., Larsen, E., and Sloth, J. 2012. Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin. **Anal Bioanal Chem.** 403: 2825–2834.

Ronkarta, S., Laurent, V., Mabon, N. and Barthe´lemy, J. 2007. Speciation of five arsenic species (arsenite, arsenate, $MMAA^{V}$,$DMAA^{V}$and AsBet) in different kind of water by HPLC-ICP-MS. **Chemosphere**. 66: 738–745.

Samanta, G., Chowdhury, T. and Chakraborti, D. 1999. Flow Injection Hydride Generation Atomic Absorption Spectrometry for Determination of Arsenic in Water and Biological Samples from Arsenic-Affected Districts of West Bengal, India, and Bangladesh. **Microchemical Journal.** 62: 174 –191.

Sasomsap, W. 1989. 2-(5′-chloro-2′-pyridylazo)-5-aminoanisole: New reagent for spectrometric determination of Rhodium(III) and Palladium(II). master of science (chemistry),major field chemistry, department of chemistry. thesis advisor : Associate Professor Pakawadee Sutthivaiyakit. Ph.D.77

Shi, J.S., Tang, Z., Jin, Z. and Jiang, G. 2003. Determination of As (III) and As (V) in soils using sequential extraction combined with flow injection hydride generation atomic fluorescence detection. **Anal. Chim. Acta**. 477: 139–147.

Shraim, A., Abu-Yousef, I., Kanan S., Abdo, N., Olszowy, H., Petry, H. and Jack NG., 2008. Quantification of Total Arsenic in Groundwater by HG-AAS Using Low Acid Concentration and L-Cysteine. **J. Int. Environmental Application & Science.** 3 (4): 215- 223.

Sigrist, M., Albertengo, A., Beldoménico, H. and Tudino, M. 2011. Determination of As(III) and total inorganic As in water samples using an on-line solid phase extraction and flow injection hydride generation atomic absorption spectrometry. **Journal of Hazardous Materials.** 188(1): 311-318.

Siripitayakunkit, U., Thaicharoen, S., and Thonghong, A. 2003. Chronic Arsenic Poisoning in Ronpiboon, Nakhon Si Thammarat,2000. **Journal of Health Science.** 12 : 89-93.

Smedley, P. and Kinniburgh, D. 2012. A review of the source, behaviour and distribution of arsenic in natural waters. **Applied Geochemistry**. 17: 517–568.

Tahir, M., Rasheed, H. and Malana, A. 2008. Method development for arsenic analysis by modification in spectrophotometric technique. **Drink. Water Eng. Sci. Discuss.** 1: 135–154.

Tuzen, M., Saygi, K., Karaman, I. and Soylak, M. 2010. Selective speciation and determination of inorganic arsenic in water, food and biological samples. **Food and Chemical Toxicology**. 48: 41–46.

Wahed, M., Chowdhury, D., Nermell, B. and Vahter, M. 2006. A Modified Routine Analysis of Arsenic Content in Drinking-water in Bangladesh by Hydride Generation-Atomic Absorption Spectrophotometry. **Journal Health Popul Nutr.** 24(1): 36-41.

APPENDIX

APPENDIX

Chemical list

Appendix Table 1 List of chemicals used in this study

Chemicals	Formula	Molecular weight	Purity	Company
Antimony(III)chloride	$SbCl_3$	291.52	99.0	Merck (Darmstadt, Germany)
Arsenic pentaoxide	H_3AsO_4 in HNO_3 0.5mol	141.94	≥99.0	Sigma-Aldrich (Steinheim, Germany)
Arsenic trioxide	As_2O_3	197.84	99.5	Sigma-Aldrich (Steinheim, Germany)
Bismuth(II)nitrate	$Bi(NO_3)_2$	1461.99	Purum	Merck (Darmstadt, Germany)
Cacodylic acid	$C_2H_7AsO_2$	138.00	≥99.0	Sigma-Aldrich (Steinheim, Germany)
Citric acid	$C_6H_8O_7$	210.14	99.5	Calro Eabra (Milan, Italy)
Disodium methyl - arsonate hexahydrate	$Na_2AsO_3CH_3.6$ H2O	291.90	97.5	Chem service (Pennsylvania, USA)

Appendix Table 1 (Continued)

Chemicals	Formula	Molecular weight	Purity	Company
Ferrour(III)chloride	$FeCl_3$	162.2	Purum	Fluka (Buchs SG, Switzerland)
Hydrazinium sulfate	$NH_2NH_2.H_2SO_4$	130.12	≥99.0	Fluka (Buchs SG, Switzerland)
Hydrochloric acid	HCl	36.50 (d=1.19 g/cm^3)	36.5	Merck (Darmstadt, Germany)
Hydroxylamine - Hydrochloride	$NH_2OH.HCl$	69.49	96.0	UNIVAR (Auckland) New Zealand
L-ascobic acid	$C_6H_8O_6$	176.13	99.0	UNILAB(Auckland) New Zealand
L-cysteine	$C_3H_7NO_2S$	121.16	Purum	Merck (Darmstadt, Germany)
Mercury(II)chloride	$HgCl_2$	271.52	Purum	Merck (Darmstadt, Germany)
Molecular sieves - 4A x 1.5 mm.	-	-	-	LOBA Chemie PVT (Mumbai India)

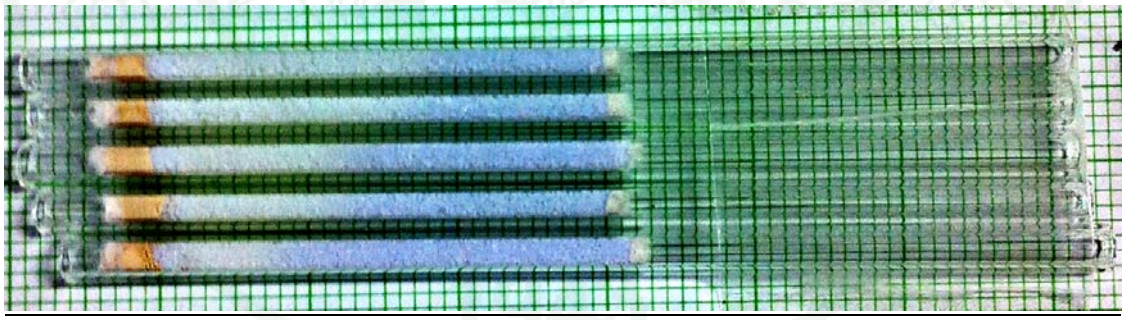
Appendix Table 1 (Continued)

Chemicals	Formula	Molecular weight	Purity	Company
Oxalic acid	$C_2H_2O_4.2H_2O$	126.07	99.0	Merck (Darmstadt, Germany)
Palladium chloride	$PdCl_2$	177.32	99.0	Fluka (Buchs SG, Switzerland)
Potassium iodide	KI	166.00	99.0	UNIVAR (Auckland) New Zealand
Potassium hydrogen-phthalate	$KOOC.C_6H_4.COOH$	204.22	99.8	UNIVAR (Auckland) New Zealand
Silica gel 60	SiO_2	60.08	-	Merck (Darmstadt, Germany)
Sodium borohydride	$NaBH_4$	37.83	≥96.0	Sigma-Aldrich (Steinheim, Germany)
Sodium hydroxide	NaOH	40.00	99.0	Merck (Darmstadt, Germany)
Sodium sulfide	Na_2S	126.04	98.0	UNIVAR (Auckland) New Zealand
Sulphamic acid	H_2NSO_3	97.09	99.5	UNIVAR (Auckland) New Zealand
Thiourea	H_2NCSNH_2	76.12	GR. Analysis	Merck (Darmstadt, Germany)
Tin dioxide	SnO_2	118.710	GR. Analysis	Fluka (Buchs SG, Switzerland)

Appendix Table 1 (Continued)

Chemicals	Formula	Molecular weight	Purity	Company
Tris(hydroxymethyl)-aminomethane	$H_2NC(CH_2OH)_3$	121.14	GR. Analysis	Merck (Darmstadt, Germany)
Zinc powder	Zn	65.39	97.0	Merck (Darmstadt, Germany)

Appendix Figure 1 Hydride generation-atomic absorption spectrometer (Spectra AA880Z,Varian).



Appendix Figure 2 Detector tube in calibration method for determination of total arsenic/As(V).

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