ANALYSIS OF ARSENIC IN GROUND WATER SAMPLES USING VOLTAMMETRY

INTRODUCTION

Arsenic is a metalloid with atomic number 33 and symbol As. It is distributed widely in the earth's crust. This element occurs naturally in a wide range of minerals and widespread uses in pigments, insecticides and herbicides represent the major source of arsenic in natural waters. The other uses of arsenic and arsenic compounds are in wood preservatives, glass manufacture, alloys, electronics, catalysts, feed additives etc. Many arsenic compounds are present in the environment and in biological system (Table 1)

Table 1 Arsenic species commonly detected in the environmental and biological systems (National research council, 1999)

Name	Abbreviation	Chemical formula
Arsenous acid	As^{III}	$As(OH)_3$
Arsenic acid	As^{V}	$AsO(OH)_3$
Monomethylarsonic acid	MMA^{V}	$CH_3AsO(OH)_2$
Monomethylarsonous acid	MMA^{III}	$CH_3As(OH)_2$
Dimethylarsinic acid	DMA^{V}	$(CH_3)_2AsO(OH)$
Dimethylarsinous acid	DMA^{III}	$(CH_3)_2AsOH$
Dimethylarsinoyl ethanol	DMAE	$(CH_3)_2AsOCH_2CH_2OH$
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$
Tetramethylarsonium ion	Me_4As^+	$(CH_3)_4As^+$
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2\ COO^-$
Arsenobetaine 2	AsB-2	$(CH_3)_3As^+CH_2\ CH_2COO^-$
Arsenocholine	AsC	$(CH_3)_3As^+CH_2\ CH_2OH$
Trimethylarsine	TMA^{III}	$(CH_3)_3As$
Arsines	AsH_3, $MeAsH_2$, Me_2AsH	$(CH_3)_xAsH_{3-x}$ (x=0–3)
Ethylmethylarsines	Et_xAsMe_{3-x}	$(CH_3CH_2)_xAs\ (CH_3)_{3-x}$ (x=0–3)
Phenylarsonic acid	PAA	$C_6H_5AsO(OH)_2$

Arsenic in small amounts may be an essential mineral (trace elements). Estimates of arsenic intakes of adults have been as low as 12-50 µg/d. Arsenic may by necessary for normal metabolism of an amino acid or protein that influences the urea cycle (Kohlmeier, 2003; Bhagvan, 2002; Mahan and Escott-Stump, 2000). Exposure to arsenic can cause a variety of adverse health effect, including skin changes and respiratory, cardiovascular, gastrointestinal and carcinogenic effect. Arsenic metabolites, especially MMA(III) and DMA(III) are extremely cytotoxic and can damage DNA directly, i.e., they are mutagenic and genotoxic. Arsenic metabolites also inhibit many enzymes with high potency (Kohlmeier, 2003). The most common types of malignancy are skin cancer, lung cancer, prostate cancer and bladder cancer. Reports of other cancers also appear: leukemia; and cancers of the breast, colon, stomach, kidney and others. Arsenic can also result in decreased production of red and white blood cells, which may cause fatigue, abnormal heart rhythm, blood-vessel damage resulting in bruising and impaired nerve function. Knowledge of the speciation of arsenic in natural water is important because the bioavailability and physiological and toxicological effects of arsenic depend on its chemical form. The arsenic occur in the natural environment in four oxidation states: As(V), As(III), As(0) and As(-III). In natural water, arsenic is predominantly present as As(III) and As(V), with a minor amount of methyl and dimethyl arsenic compound being detected (Figure 1)

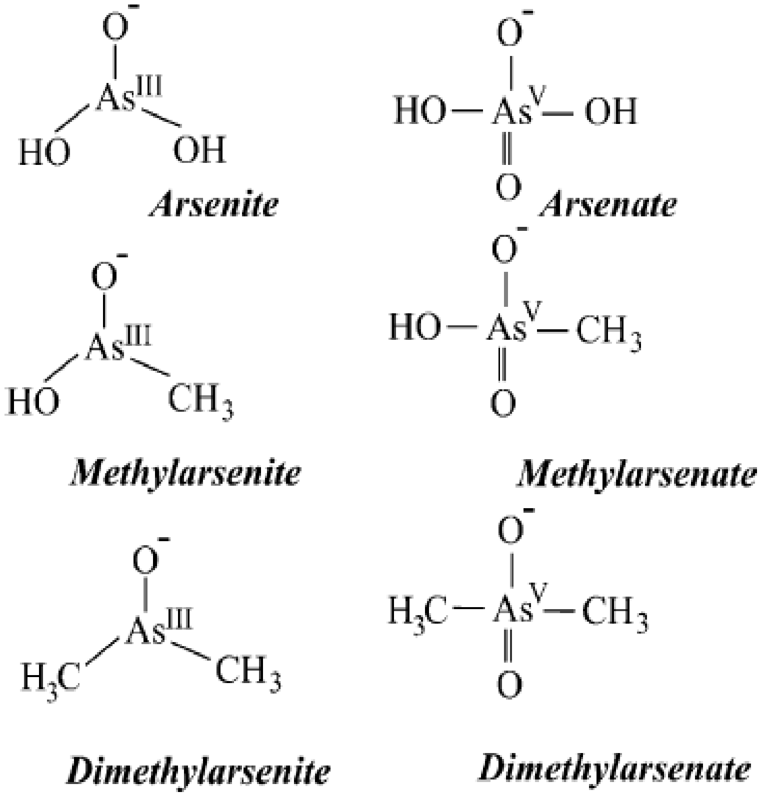

Figure 1 Arsenic species found in water (Hung *et al.*, 2004)

Arsenic(III) is more toxic than arsenic(V). Toxicities of other arsenic species such as monomethylarsenic acid and dimethylarsenic acid are lower than the inorganic arsenic species. For this reason the US Environmental Protection Agency (EPA) and the World Health Organization (WHO) recommend the maximum contaminant level (MCL) of 10 μgL^{-1} for inorganic arsenic in drinking water and have set a provisional tolerable weekly intake (PTWI) of 15 μgkg^{-1} body weight, which is equivalent to a tolerable daily intake (TDI) of 150 μg for a 70 kg adult and of 64 μg for a 30 kg child.

Arsenic toxicity problems were first recognized in Ron Piboon District, Nakhon Si Thammarat Province, which locate in southern part of Thailand in 1987. Such problems were largely attributable to contaminated water. The source of arsenic derived from mineral separation at former bedrock tin mining sites disseminated arsenopyrite-rich waste generated by the informal mining sector, arsenic-rich waste generated by two ore-dressing plants near Ron Piboon town and extensive alluvial placer tin deposits. These facts indicate why it would be of priority interested to develop methods for the selective determination of arsenic(III).

The techniques used for the detection of arsenic species in environmental and biological sample should be sensitive and selective. Species instability during sampling, storage and sample pretreatment are all very important that must be considered. Therefore the rapid analysis of samples to prevent species conversion is also important. Inductively coupled plasma-mass spectrometry (ICP-MS) has become a favored detection technique in arsenic analysis. It provides ultra-sensitivity, multi-element capability and can be combined with the separation techniques for speciation analysis. The multi-element capability allows for the simultaneous determination of different elements in addition to arsenic. Another common technique used in arsenic speciation is hydride generation (HG). HG allows for extremely low detection limits. However, not all arsenic species form hydrides, and decomposition techniques are usually required. Furthermore other methods to determine arsenic have been used: ultraviolet spectrometry; atomic absorption spectrometric methods (AAS); atomic emission spectrometry (AES), generally with inductively coupled plasma (ICP-AES); X-ray spectrometry and neutron-activation analysis (NAA). Methods based on these techniques require expensive instrumentation, complicated procedures and special sample pre-treatment. Besides, most of these methods are essentially sensitive to total arsenic.

In general, electrochemical methods offer possibilities to determine arsenic and arsenic compounds at low concentrations. These techniques offer advantages over other methods as follows: simple instrumentation and operation, low cost, high sensitivity and excellent selectivity which allow diversifying the oxidation states of arsenic featuring different level of toxicity.

Literature reports many publications which describe the use of stripping voltammetry, both in the cathodic and anodic modes, for arsenic analysis in water systems. Cathodic stripping voltammetry (CSV) and cathodic stripping potentiometry (CSP) for arsenic determination in natural waters are usually carried out at a hanging mercury drop electrode (HMDE) or at a mercury film electrode (MFE) in the presence of copper ions (Greulach and Henze, 1995; Adeloju *et al.*, 1999). Arsenic(V) is electroinactive at the mercury surface, therefore a prior reduction step to arsenic with D-mannitol or cysteine is necessary.

Anodic stripping voltammetry (ASV) for arsenic measurement is usually performed at a gold disk or gold-coated electrode in many types of samples (Huang *et al.*, 1999; Kopanica and Nocotny 1998; Sun *et al.*, 1997; Švancara *et al.*, 2002)

The objective of this research is to develop method for determination of arsenic(III) in ground water samples by differential pulse anodic stripping voltammetry (DPASV) and to use the artificial neural network to predict the arsenic(III) contents in ground water samples from Ron Piboon District, Nakhon Si Thammarat Province. The results of determination of arsenic(III) is compared with hydride generation-atomic absorption spectrometry (EPA Method 1632).

LITERATURE REVIEWS

1. Literature reviews of voltammetry for determination of arsenic

Various electrochemical techniques have been used for the determination of arsenic at trace level such as polarography, anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV). Among electrochemical techniques, the anodic stripping voltammetry is one of the most sensitive of all methods and offers a promising technique to investigate trace arsenic. The conditions and applications of each technique were reviewed as follows.

Adeloju *et al.* (1999) determined arsenic(III) by cathodic stripping potentiometric (CSP) on a glassy carbon mercury film electrode in 1 M HCl which contained copper(II) ions. Optimum determination of arsenic was accomplished with an electrolysis potential of -600 mV vs Ag/AgCl (1M HCl), an electrolysis time of 30 s and application of a constant cathodic stripping current of -1.5 μA. As little as 2 μgL^{-1} of arsenic can be detected by this method in the presence of 5 mgL^{-1} copper(II) ion. The arsenic(III) peak area decreased slightly when higher copper(II) concentrations (>7 mgL^{-1}) were added because a copper-arsenic intermetallic compound is formed during the deposition step. A linear concentration range of 10-200 μgL^{-1} was achieved under these conditions. Interferences from organic and inorganic substances were successfully overcome by the use of dry ashing with sulfuric acid as ashing aid and L-cysteine as a reducing agent to reduce arsenic(V) to arsenic(III). This method was used to determination of arsenic in water, bovine liver and dogfish muscle samples.

Billing *et al.* (2002) determined small quantities of arsenic in pure gold samples using matrix exchange differential pulse stripping voltammetry (DPSV) at a gold film electrode. A wall-jet cell (WJC) and an on-line deoxygenation system were used to facilitate matrix exchange. The formation of the gold(I) cyanide complex prevented gold from depositing on the electrode simultaneously with the arsenic. It was found that using alkaline solutions produced interference from gold(I) cyanide and also led to the passivation of the gold film electrode due to the presence of uncomplexed cyanide. Thus, the sample solutions were adjusted to a pH of 3. Matrix exchange electrolytes consisting of 4 $molL^{-1}$ hydrochloric acid or a combination of 2 $molL^{-1}$ sulfuric acid and 0.2 $molL^{-1}$ hydrochloric acid could be utilized. Arsenic concentrations as low as 0.1 mg L^{-1}, could readily be detected in a gold matrix with a 60 s deposition time. Cobalt and silver did not interfere with the arsenic determination as the present in relatively low concentrations in a gold sample, but copper interfered even when present at similar concentrations to that of arsenic.

Dai *et al.* (2004) developed novel method for the detection of arsenic(III) in 1 M HCl at a gold nanoparticle-modified glassy carbon electrode. The gold nanoparticles were electrodeposited onto the glassy carbon electrode via a potential step from +1.055 to -0.045 V vs saturated calomel reference electrode(SCE) for 15 s from 0.5 M H_2SO_4 containing 0.1 mM $HAuCl_4$. The resulting electrode surfaces were characterized with both atomic AFM and cyclic voltammetry. Anodic stripping voltammetry of arsenic(III) on the modified electrode was performed. Linear sweep voltammetry (LSV) and square wave voltammetry (SWV) were compared, with 1 M HCl serving as the supporting electrolyte. In the two methods were using depositions potential at -0.3 V vs SCE for 180 s. The arsenic stripping peaks were seen between + 0.1 and + 0.2 V. The limit of detections of 0.0096 ppb using LSV and 0.014 ppb using SWV are achieved. This method was investigated on the detection of arsenic(III) in spiked river water.

Dugo *et al.* (2005) developed a sensitive and accurate method for speciation of inorganic arsenic in alimentary and environmental aqueous samples by using derivative anodic stripping chronopotentiometry (dASCP) at a gold film electrode. A 3 M HCl solution was using as the stripping medium. Arsenic(III) was directly determined in the aqueous matrix at a deposition potential at -0.3 V vs Ag/AgCl, the electrolysis time was 180 s and at a anodic constant current of 2.5 μA, without any sample pre-treatment. Moreover time consuming de-oxygenation step prior to the analysis was not necessary. The direct measurement of arsenic(V) required a deposition potential more negative than -1.6 V and a deposition time longer than 300s, the result in measurements were obtained after reduction of arsenic(V) to arsenic(III) with KI in 3 M KCl. Under the optimized conditions, the obtained limit of detection and limit of quantification were 0.080 μgL^{-1} and 0.25 μgL^{-1}, respectively. This method is applied for determination of arsenic(III) and total inorganic in seawater, tap water, mineral water and commercial beverages.

Ensafi *et al.* (2004) studied method for simultaneous determination of trace levels of copper(II) and molybdenum(VI). The method is based on the reduction of complexing of copper(II) and molybdenum(VI) with pyrogallol red (PGR). The complexes of these metal ions are analyzed by cathodic stripping differential pulse voltammetry based on the adsorption collection of the complexes onto a hanging mercury drop electrode (HMDE). Supporting electrolyte was used Briton–Robinson buffer solution, pH 2.5, and 2 M sodium chlorate solution. The optimum conditions using adsorption potential of 0.00Vfor 90 s, a differential pulse voltammogram was recorded from +0.20 to −0.40V. The calibration plot of copper(II) and molybdenum(VI) was linear over the concentration range of 2.0–70.0 $ngmL^{-1}$ and 0.8–80.0 $ngmL^{-1}$, respectively. The detection limit of 0.3 $ngmL^{-1}$ for copper(II) and 0.1 $ngmL^{-1}$ for molybdenum(VI) were obtained. The relative standard deviation of 5 $ngmL^{-1}$ for copper(II) and molybdenum(VI) were 0.8 and 1.2%, respectively.

This method was applied to determine copper(II) and molybdenum(VI) in river water, tap water and alloy.

Ferreira and Barros (2002) developed method for the determination of arsenic(III) and total inorganic arsenic in natural spring and mineral water by square wave cathodic stripping voltammetry (SWCSV) at a hanging mercury drop electrode (HMDE). In the determination of arsenic(III), pre-concentration was carried out on the electrode from a 1 M HCl solution in the presence of 45 ppm of copper(II) at a potential of -0.39 V vs Ag/AgCl. In the determination of total inorganic arsenic used sodium thiosulfate to reduce arsenic(V) to arsenic(III) and the pre-concentration was carried out in 1 M HCl in the presence of 400 ppm of copper(II) at a potential of -0.40 V vs Ag/AgCl. For determination of arsenic(III) and total inorganic arsenic the detection limits were 0.06 ppb and 0.7 ppb, respectively. The quantification limit of arsenic(III) was 0.2 ppb for a deposition time of 40 s. The relative standard deviation (RSD) was calculated to be 6 % (n=13) for 8 ppb of arsenic(III). For total inorganic arsenic, the quantification limit was 2 ppb for a deposition time of 3 min. The RSD was calculated to be 3 % (n=10) for 8 ppb of arsenic(V). The value for total arsenic correlated well with that obtained by optical emission spectrometry with ICP coupled to hydride generation (OES-ICP-HG).

Greulach and Henze (1995) reported on analysis of arsenic(V) by cathodic stripping voltammetry in a mannitol containing perchloric solution. It is based on the coprecipitation of arsenic(V) with copper(II) and determination by further reduction to AsH_3 at the hanging mercury drop electrode. Optimum determination of arsenic was accomplished with an accumulation potential of -0.55 V, an accumulation time of 60 s. The detection limit was 4.4 μgL^{-1} arsenic(V) and the determination limit was 11.0 μgL^{-1}. No significant interferences have been applied to the analysis of arsenic in standard stream sediment and in water samples.

He *et al.* (2004) studied a simple, fast and sensitive speciation method for determination of inorganic arsenic compounds in natural water samples at the trace level based on differential pulse cathodic stripping voltammetry (DPCSV) at a hanging mercury drop electrodes (HMDE). A 1 M HCl solution was using as the supporting electrolyte. DPCSV was performed using a deposition potential of -0.44 V vs Ag/AgCl reference for 60 s. Arsenic(III) was deposited as $Cu_xSe_yAs_z$ intermetallic compound on a mercury electrode. Determination of total arsenic is performed by reducing arsenic(V) to arsenic(III) using sodium meta-bisulfite/sodium thiosulfate reagent stabilized with ascorbic acid. Arsenic(V) is quantified by difference between total arsenic and arsenic(III) concentrations. The detection limit (S/N > 3) was 0.5 μgL^{-1} with a linear range from 4.5 to 180 μgL^{-1}. The relative standard deviation (n = 6) was 2.4, 2.5, 4.2 % for arsenic(III) and 8.0, 6.8, 9.0 % for arsenic(V) at level of 45, 10 and 5 μgL^{-1}, respectively. Results obtained on several natural water samples analyzed both in laboratory and on-site compared well with those obtained by high resolution (HR) ICP-MS, GFAAS and IC-AFS.

Henze *et al.* (1997) determined arsenic(V) and arsenic(III) by cathodic stripping voltammetry in a mannitol-sulfuric acid medium. The arsenic was coprecipitated with copper(II) and selenium(IV) and reduced to arsine at the hanging mercury drop electrode(HMDE). Using an accumulation potential of -0.55 V, an accumulation time of 240 s. The determination limit was calculated by the calibration curve and was found to be 0.93 μgL^{-1}. The detection limit was estimated to be 0.52 μgL^{-1}. For the speciation of arsenic(III) and arsenic(V), it was preferred to determine arsenic(III) first, using the recommended supporting electrolyte but omitting the mannitol. This method was applied to the quantification of arsenic in spiked distilled water and several different natural fresh water samples.

Huang and Dasgupta (1999) offered an inexpensive portable stripping voltammetric instrument with a simple procedure for the field analysis of arsenic(III) and arsenic(V) using gold film on platinum wire electrode. The instrument was operated in a desktop or a notebook computer, equipped with an A/D-D/A card. Arsenic(III) was first determined at a deposition potential of -0.2 V. Measurement of arsenic(V) involved complete oxidation using $KMnO_4$ or bromine-water, followed by electrolysis at -1.6 V. The stripping signal increased linearly with arsenic(III) concentration up to 350 μgL^{-1} with r^2 = 0.9984 and 60 μgL^{-1} with r^2 = 0.9986 for deposition time of 5 and 20 s, respectively. The limit of detection was calculated to be 0.5 μgL^{-1}. Reproducibility was also demonstrated with a relative standard deviation of 2.2% for several measurements in 10-60 μgL^{-1} arsenic(III).

Kamenev *et al.* (2005) studied the simultaneous determination of arsenic(III) and copper(II) by anodic stripping voltammetry in a mixed EDTA-phosphoric acid supporting electrolyte at a gold-graphite electrode. Different techniques were proposed for the isolation of the analytical signals from the total voltammetric signal using postelectrolysis and subtracting voltammograms. The arsenic(III) peak current was linear in the range from 0.013 to 0.067 μgmL^{-1}. The effect of the copper(II) concentration on arsenic(III) peak was studied in the range of copper(II) concentrations from 0.05 to 0.35 μgmL^{-1}. The proposed techniques allowed arsenic(III) to be determined in the presence of 30-fold amounts of copper(II) and copper(II) to be determined in the presence of 40-fold amounts of arsenic(III).

Kopanica and Novotný (1998) determined arsenic(III) in aqueous solutions by differential pulse anodic stripping voltammetry using disc gold electrode. The linear range was obtained in the range between 0.2 and 250 μgL^{-1} of arsenic(III) and the detection limit was 0.15 μgL^{-1}. The good reproducibility of measurements was achieved by a programmed electrochemical treatment of the electrode surface before each measurement. It has been found that the presence of Triton X-100, used as a model substance of naturally occurring surface active compounds, positively influenced the nature of the arsenic(III) dissolution peak which make peak current became more symmetric and slightly increased and moved the peak potential shifted to more positive potentials.

Li and Smart (1996) reported on sub-nanomolar concentration of arsenic(III) in natural waters using square wave cathodic stripping voltammetry (SWCSV) at a hanging mercury drop electrode(HMDE). Pre-concentration was carried out in 2M HCl in the presence of 0.8 nM $CuCl_2$ at a potential of -0.4 V vs Ag/AgCl. The deposited intermetallic compound (Cu_xAs_y) was reduced to copper at a potential of about -0.8 V vs Ag/AgCl. The deposition potential and time, the hydrochloric acid and copper concentrations on the stripping signal, were all optimized. The detection limit was approximately 0.06 nM (5 parts per trillion) when 10 min deposition time was used. The relative standard deviation was calculated as 8% (n=11) at 1×10^{-9} M arsenic for 1 min period of deposition. This methodology was employed in the analysis of arsenic in river and sea water samples.

Locatelli and Tori (2000) suggested a new speedy, precise and accurate analytical procedure for the simultaneous determination of heavy metals by differential pulse cathodic stripping voltammetry (DPCSV) for arsenic(III). and selenium(IV), and anodic stripping voltammetry for copper(II), lead(II), cadmium(II), zinc(II) and manganese(II) at a stationary mercury electrode. The ammonia-ammonium chloride buffer pH 9.4 was using as the supporting electrolyte. The analytical procedure was verified by the analysis of the standard reference materials: Sea Water BCR-CRM 403; Lagarosiphon Major BCR-CRM 060; and Cod Muscle BCR-CRM 422. In all cases, the precision, expressed as relative standard deviation (s_r) and the accuracy, expressed as relative error (e), were lower than 5%. Limit of detection of each element were approximately 10^{-9} M. This method was applied to the determination of heavy metals in complex environmental aquatic matrices involved in the food chain such as sea water, algae and fishes.

Locatelli and Torsi (2002) determined arsenic(III), selenium(IV), copper(II), lead(II), cadmium(II), zinc(II) by differential pulse cathodic stripping voltammetry (DPCSV) for arsenic(III) ,selenium(IV) and differential pulse anodic stripping voltammetry (DPASV) for copper(II), lead(II), cadmium(II), zinc(II) at stationary mercury electrode. The voltammetric measurements were carried out using the ammonia-ammonium chloride buffer pH 9.2 as supporting electrolyte. The optimal conditions for arsenic(III) and selenium(IV) were accomplished with a deposition potential of -1.050 for 230 s. DPASV measurements of copper(II), lead(II), cadmium(II), zinc(II) were based on deposition potential of -1.300 for 180 s. Bath the precision and the accuracy were less than 5%. This procedure was utilized for the monitoring of heavy metals in sea water, sediments, algae and clams.

Locatelli and Torsi (2003) reported on an analytical procedure for simultaneous determination of copper(II), lead(II), cadmium(II), zinc(II) and antimony(III) at trace and ultratrace level in matrices involved in foods and food chain as whole meal, wheat and maize meal. Differential pulse anodic stripping voltammetry (DPASV) was employed for simultaneously determined all the elements, using a stationary hanging drop mercury electrode (HDME) and 0.5 M HCl as supporting electrolyte. The optimum conditions using electrolysis potential of -1.150 V for 210 s. The precision as repeatability, expressed as relative standard deviation and the accuracy, expressed as relative error were 3 and 6%, respectively. The limits of detection were in the range 0.009 - 0.096 $\mu g g^{-1}$.

Profumo *et al.* (2005) determined arsenic(III) and total inorganic arsenic at ppb and sub ppb level by using cathodic stripping voltammetry at hanging mercury drop electrode (HMDE). This method is based on the formation of a copper-arsenic intermetallic at HMDE during the preconcentration step. Sodium dithionite was the best reducing agent to reduce arsenic(V) to arsenic(III) and then arsenic(III) was determined in HBr supporting electrolyte. The best sensitivity is obtained at deposition potential equal to -0.4 V for 1 min. Quantification limits of 0.010 and 0.020 ppb for arsenic(III) and arsenic(V), respectively are obtained. Standard deviation for 1 ppb arsenic concentration (n=7) is 5%. The presence of organic species of arsenic in the samples does not interfere with the determination of inorganic arsenic(III) and arsenic(V). This method was applied to thermal, spring and sea waters.

Rasul *et al.* (2002) developed method for routine measurement and speciation of inorganic arsenic in ground water by using anodic stripping voltammetry (ASV) at gold film coated glassy carbon electrode. In this method, the following values for the parameters were used: deposition potential -150 mV for 60 s, scan rate 50 mVs^{-1}. The measurement of total inorganic arsenic was done by reducing arsenic(V) to arsenic(III) with $SO_2(g)$ produced by the acid decomposition of Na_2SO_3. The analytical performance of this method can be summarized as follows: the relative standard deviation (RSD) of three replicate was found to be 10% for 10 $\mu g L^{-1}$, the detection limit was 1.2 $\mu g L^{-1}$ at 95% confidence limits at 120 s deposition time. This method was used for determination of inorganic arsenic in groundwater in Bangladesh.

Sadana (1983) determined arsenic in drinking water contaminated with copper(II) by differential pulse cathodic stripping voltammetry (DPCSV). In this method, the sample was acidified to 7.5 N HCl and arsenic was perconcentrated on a hanging mercury drop electrode. The optimized operating parameters were used initial potential at -0.6 V for 120 s. A chemical prereduction of arsenic(V) to arsenic(III) by hydrobromic acid is essential for the determination of total arsenic. The relative standard deviation (RSD) of ten replicate measurements on a sample containing 10 ng of arsenic(III)mL^{-1} was 6.4 %. The detection limit under the experimental conditions was 1 ngmL^{-1} arsenic. The accuracy of the method was tested by analysis of the EPA reference water, series 575. The result gave a good agreement with the certified values.

Sancho *et al.* (1998) determined copper(II) and arsenic(III) at the μgkg^{-1} level by anodic stripping voltammetry for copper and cathodic stripping voltammetry for arsenic(III) in the differential-pulse mode (DPASV and DPCSV) at a hanging mercury drop electrode (HMDE). The optimal condition for copper was accomplished with a deposition potential of -0.8 V for 30 s. DPCSV measurements of arsenic were based on its accumulation onto the HMDE as an intermetallic Cu-As compound followed by the reduction of As^0 to arsine in hydrochloric acid medium. The limits of detection of copper(II) and arsenic(III) were estimated to be 2.5 μgkg^{-1} and 2 μgkg^{-1}, respectively. The method was applied to the determination of copper(II) and arsenic(III) in refined beet sugar samples.

Stoytcheva *et al.* (1998) studied the inhibition action of arsenic(III) on the activity of the enzyme acetylcholinesterase immobilized on the surface of a graphite electrode by using chronoamperometry. The arsenic(III), interacting with the acetylcholinesterase, inactivates this enzyme; the quantity of the thiol generated diminishes; the value of the registered oxidation current also decreases as a function of the AsO_3^{3-} concentration. The experiments were performed in a Britton-Robinson buffer solution. The optimum conditions for the analyses were pH 7 and an acetylthiocholine iodide concentration 1x10^{-3} molL^{-1}. The relative standard deviation (RSD) and the detection limit of this method were found to be 0.33 and 2x10^{-10} molL^{-1} AsO_3^{3-}, respectively. The obtained results were used to construct a calibration curve of inorganic arsenic determination in the range of 2x10^{-10} to 1x10^{-6} molL^{-1}. This method was applied for estimating the arsenic contents in industrial waters after preliminary elimination of heavy metal ions which were inhibitors of the acetylcholinesterase.

Sun *et al.* (1997) reported on a new method of efficient rotating gold-film glassy-carbon electrode preparation for the anodic stripping voltammetry (ASV) determination of arsenic(III) and arsenic(V) in seawater. The procedure called for a rotating glassy carbon electrode with a gold film deposited from a gold plating solution. For optimum reproducibility, the electrode was re-plated between each measurement and the electrode was washed with the sample before each measurement. Other factors likely to affect the stability of electrode response and method sensitivity, including: acidity(pH), deposition time and potential, rotation and scan rate, electrode reaction and the reduction step of arsenic(V) to arsenic(III), were also investigated. Electroinactive arsenic(V) was reduced to arsenic(III) by gaseous SO_2 prior to ASV determination. For a deposition time of 4 min the limit of detection (LOD) was approximately 0.19 μgL^{-1} for arsenic(III). Precision of the proposed method was very good (RSD = 2-0.6% at 1-5 μgL^{-1}). The new gold film procedure was implemented in the detection of arsenic in seawater samples.

Švancara *et al.* (2002) developed method for determination of arsenic in water samples with a gold film-plated carbon paste electrode using constant current stripping analysis (CCSA). The optimized medium contained both perchloric and hydrochloric acid in highly acidic medium ($pH \rightarrow 0$). The accumulation step was initiated with conditioning the electrode at -0.4 V for 15 s and stripping from -0.4 to +0.6 V with a constant current of +5 μA. The limit of detection of 2 μgL^{-1} arsenic(III) and of approximately 0.5 μgL^{-1} arsenic(V) were estimated. This method was used to determine arsenic(III), arsenic(V) and total arsenic in samples of polluted river water.

Tanaka and Sato (2001) determined arsenic and antimony in iron and steel by differential pulse anodic stripping voltammetry at a rotating gold film electrode in hydrochloric-nitric acid mixture without any preliminary separation of the matrix. Arsenic(V) and antimony(V) were reduced to the trivalent state with potassium iodide prior to the voltammetric measurements. Antimony(III) alone in the sample solution was first deposited on a rotating gold film electrode at -0.25V vs SCE for 180 s, the electrode potential was scanned from -0.25 to 0.2V vs SCE at a rate of 40 mVs^{-1}. Next, both arsenic and antimony were deposited at -0.45V vs SCE in another sample solution and the peak area was measured by differential pulse anodic stripping. The arsenic concentration was calculated by difference. The interference of iron(III) was eliminated by reducing it to iron(II). The relative standard deviations (RSDs) for five measurements of 25 $ngcm^{-3}$ of arsenic and antimony were 3.4 and 1.7%, respectively. The detection limits for the analytes were 2.1×10^{-8} and 3.4×10^{-9} $moldm^{-3}$, respectively.

Zima and van den Berg (1994) determined arsenic(III) and total arsenic in sea water by differential pulse cathodic stripping voltammetry at a hanging mercury drop electrode in the presence of pyrrolidine dithiocarbamate (PDC). Deposition of arsenic(III) on the HMDE was carried out for 1 min at -0.3 V, whereas the scan was initiated from 0 V towards more negative potentials. Total arsenic was determined after chemical reduction of arsenic(V) to arsenic(III) by sulfur dioxide. Optimized conditions include a PDC concentration of 0.5 μM and a pH ≈ 0.8. The detection limit of the method was 3 nM arsenic. The method was tested by analysis of CASS-2 certified sea water. This method was applied to determine arsenic in sea water originating from the North Atlantic.

2. Literature reviews of hydride generation-atomic absorption spectrometry(HG-AAS) for determination of arsenic

Hydride generation-atomic absorption spectrometry (HG-AAS) was a general technique used for determination of arsenic. Additionally, it was standard method used in EPA method.

Anthemidis *et al.* (2005) developed a simple and robust on-line sequential insertion system coupled with hydride generation atomic absorption spectrometry (HG-AAS) for determination of arsenic(III) and total inorganic arsenic without pre-reduction step. The proposed manifold, which was employing an integrated reaction chamber/gas–liquid separator (RC-GLS), was characterized by the ability of the successful managing of variable sample volumes (up to 25 ml), in order to achieve high sensitivity. Arsine was able to be selectively generated either from inorganic arsenic(III) or from total arsenic, using different concentrations of HCl and $NaBH_4$ solutions. For 8 ml sample volume consumption, the sampling frequency was 40 h^{-1}. The detection limits were 0.1 and 0.06 μgL^{-1} for arsenic(III) and total arsenic, respectively. The precision (relative standard deviation) at 2.0 μgL^{-1} (n = 10) level were 2.9 and 3.1% for arsenic(III) and total arsenic, respectively. The accuracy was evaluated, by determining the arsenic concentration of a certified reference material NIST CRM 1643d. The recovery obtained was 96%. This method was also applied to the analysis of local natural water samples including river, lake and tap water.

Cabredo *et al.* (1998) studied a cold trap system for the simultaneous determination of arsenic, antimony, selenium and tin by continuous hydride generation and gas phase molecular absorption spectrometry. The hydride generation was carried out in two steps: first, tin hydride was generated at 0.05M HCl and second step, arsenic, antimony and selenium hydrides were generated at 2 M HCl. All hydrides were collected in a liquid nitrogen cryogenic trap and transported to the flow cell of a diode array spectrophotometer, where molecular absorption spectra were obtained in the

190-250 nm range. Resolutions of five calibration solution were carried out by using multiple linear regression analysis (MLR). Under the optimum conditions were found and using the wavelengths of maximum sensitivity (190, 198, 220 and 194 nm), the analytical characteristics of each element were calculated. The detection limits were 0.050, 0.020, 0.12 and 1.1 μgmL^{-1} and quantification limits were 0.050, 0.050, 0.20 and 1.5 μgmL^{-1} for arsenic, antimony, selenium and tin, respectively.

Coelho *et al.* (2002) developed method for the direct determination of arsenic(III) and arsenic(V) in water samples by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS), without pre-reduction of arsenic(V). The flow injection system was operated in the merging zones configuration where sample and $NaBH_4$ were simultaneously injected into two carrier streams, HCl and H_2O, respectively. The carrier streams (1.0 molL^{-1} HCl and H_2O) solutions were pumped at 3.6 mLmin^{-1}. The $NaBH_4$ concentration of 0.1% (w/v) was selected in order to obtain the best analytical performance arsine selective generation from arsenic(III). The total arsenic concentration was determined in the same flow injection system using a $NaBH_4$ concentration of 3.0% (w/v). Arsenic(V) was calculated as the difference between total arsenic and arsenic(III). The detection limits were 0.3 μgL^{-1} for arsenic(III) and 0.5 μgL^{-1} for arsenic(V). The relative standard deviations were 2.3% for 0.1 mgL^{-1} arsenic(III) and 2.0% for 0.1 mgL^{-1} arsenic (V). The developed method was applied for the quantification of arsenic in water samples.

EPA method 1632. (1998) reported method for determination of total inorganic arsenic, arsenic(III), arsenic(V), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in filtered and unfiltered water by hydride generation quartz furnace atomic absorption spectrometry. Water samples were collected directly into cleaned linear polyethylene bottles using sample handling techniques specially designed for collection of metals at trace levels. Water samples were preserved in the field by the addition of 3 mL of pretested 6M HCl per liter of sample. Four percent $NaBH_4$ solution was used to convert inorganic arsenic, MMA and DMA to volatile arsines. Arsines were purged from the sample onto a cooled glass trap packed. The trapped arsines were thermally desorbed, in order of increasing boiling points, into an inert gas stream that carries them into the quartz furnace of an atomic absorption spectrophotometer for detection. This method was designed for measurement of total arsenic and arsenic species in water (range 0.01-50 μgL^{-1} for total arsenic and arsenic species). The detection limits and quantitation levels in this method are usually dependent on the level of background elements rather than instrumental limitations. The method detection limits (MDL) for total inorganic arsenic, arsenic(III), and arsenic(V) have been determined to be 3 ngL^{-1} when no background elements or interferences were present. The minimum level (ML) has been established at 10 ngL^{-1}. The method detection limits for MMA and DMA were about 15 ngL^{-1} and the minimum levels were about 45 ngL^{-1}.

Flores *et al.* (2001) studied the method to minimize the interference of volatile nitrogen oxides from digestion procedures with nitric acid on the determination of arsenic by hydride generation atomic absorption spectrometry (HG-AAS). Sulfamic acid (SA) was proposed to minimize this interference by employing three procedures for the digestion of hair in closed systems: conventional and microwave (MW) heating in polytetrafluoroethylene (PTFE) vessels and by microwave heating in glass vials. After dilution, arsenic was determined by HG-AAS. The conditions for determination of arsenic were 10 mL of 3 molL^{-1} HCl, 15 gL^{-1} $NaBH_4$ and 30 s for purge time. The spike recoveries were better than 94% for all digestion procedures and the results for the certified hair reference material were well within the 95% confidence interval of the certificate.

Flores *et al.* (2002) presented the method for the determination of arsenic by batch hydride generation atomic absorption spectrometry (HG-AAS) in commercial samples of injectable drugs containing high concentrations of Sb(V) as N-methylglucamine antimonite. The following experimental conditions for the determination of total arsenic, as arsenic(V), were 10% (m/v) citric acid, 1.5% (m/v) sodium tetrahydroborate solution and 30 s for purge time. An interference study was carried out to make sure that these elements have no influence on the determination of arsenic(V) by the proposed batch HG AAS procedure. For Cu and Bi a small decrease was observed with masses higher than 10 and 100 mg, respectively. No interference on arsenic(III) and arsenic(V) signals was observed in the presence of Sb(V) up to 1 mg. The tolerance limits for Ni(II), Cu(II) and Pb(II) were 1 mg, 100 mg and 100 mg, respectively. The detection limit (3σ) was 0.8 ng for total arsenic.

Karthikeyan *et al.* (1999) studied a sorbent extraction hydride generation-flow injection analysis atomic absorption spectrometric (HG-FIAS-AAS) method for determination of arsenic(III) and arsenic(V) based on online preconcentration on a microcolumn packed with activated alumina. Samples are complexed with quinolin-8-ol-5-sulphonic acid solutions in online flow injection system and adsorbed on the column. A 10% HCl and 0.5% sodium borohydride were used for elution. A standard HG-FIAS system was used with a carrier gas flow rate of 75 mLmin^{-1}. The sorption/elution was highly reproducible giving precision of 3.6 and 5.0% for arsenic(III) and arsenic(V), respectively. With a preconcentration time of 20s, 12 and 10-fold enhancement in sensitivity was obtained for arsenic(III) and arsenic(V) respectively, allowing a sample frequency of 60 h^{-1}. The respective detection limit were 0.05 and 2 ng mL^{-1} for arsenic(III) and arsenic(V). The method has been applied to sea water samples.

Martín *et al.* (1995) developed an online high-performance liquid chromatographic-microwave assisted oxidation-hydride generation atomic absorption spectrometric (HG-AAS) system for determination of arsenic(III), arsenic(V), dimethylarsinate(DMA), monomethylarsonate(MMA), arsenobetaine (AsB) and arsenocholine (AsC) in environmental samples. Ion-pair reversed-phase chromatography using tetrabutylammonium phosphate (TBAP) as the mobile phase and anion-exchange chromatography were evaluated. The best chromatographic conditions for the Hamilton PRP-X 100 column were pH 6.0 or 10.7, flow rate 2 mLmin^{-1} and 12 mmolL^{-1} phosphate buffer concentration for determining the six arsenic species, since AsB and AsC can be independently determined by taking the difference between measurements at pH 6.0 and 10.7. The detection limits were 97-143 and 10-30 μgL^{-1} for ion-pair reversed-phase and anion-exchange chromatography, respectively. The proposed methods were applied to water samples and sediments samples.

Mester and Fodor (1996) developed method for determination of arsenic(III), monomethylarsonate (MMAs), dimethylarsinate (DMAs), arsenic(V) from waters by using ion-pair chromatography hydride generation and atomic fluorescence spectrometry. The analytical column was a C_{18} bonded silica column modified by didoctyldimethylammonium bromide (DDAB). The optimal hydrogen chloride and sodium borohydride quantity for hydride generation were 2% (w/v) of $NaBH_4$ and 1.5 mol/dm^3 of HCl. The detection limit of the developed method, by using a 250-mm^3 loop, was 0.4 ng/cm^3 for arsenic(III), 0.8 ng/cm^3 for DMAs, 0.6 ng/cm^3 for MMAs and 1.2 ng/cm^3 for arsenic(V). Responses for all the four arsenic species tested were linear in 10-3000 ng/cm^3 range, based on five replicate measurements of each solutions of four species at eight different concentrations.

Niedzielski (2005) reported on the new concept of hyphenated analytical system: simultaneous determination of inorganic arsenic(III), arsenic(V), selenium(IV) and selenium(VI) by high performance liquid chromatography-hydride generation-(fast sequential) atomic absorption spectrometry during single analysis (HPLC-HG-AAS). The separation of inorganic speciation forms of arsenic and selenium were performed on an anion-exchange column Supelco LC-SAX1. Phosphate buffer at pH 5.40 was used as a mobile phase. Separation of inorganic speciation forms of arsenic and selenium within an element stream reaching the hydride generation system enables determining all of these forms: arsenic(III), arsenic(V), selenium(IV) and selenium(VI) during a single analysis using a fast sequence mode of the AAS spectrometer. The obtained detection limits were 7.8 ngmL^{-1} for arsenic(III); 12.0 ngmL^{-1} for arsenic(V); 2.4 ngmL^{-1} for selenium(IV) and 18.6 ngmL^{-1} for selenium(VI) and precision 10.5%, 12.1%, 14.2% and 17.3%, respectively, for 100 ngmL^{-1}. The method was applied for ground water analysis.

Nielsen and Hansen (1997) presented the method for the determination and speciation of trace inorganic arsenic, arsenic(III) and arsenic(V) by flow injection-hydride generation atomic absorption spectrometry (FI-HG-AAS). The determination of total arsenic was obtained by online reduction of arsenic(V) to arsenic(III) by means of 0.50 % (w/v) ascorbic acid and 1.0 % (w/v) potassium iodide in 4 M HCI. The temperature of the quartz tube atomizer cell was set at 900°C. The combined sample and reduction solution was initially heated by flowing through a knotted reactor immersed in a heated, thermostatted oil bath at 140°C, and subsequently, for cooling the reaction medium, a knotted reactor immersed in a water bath at 10°C. By using the volume-based FI-HG-AAS system with the heating and cooling reactors removed, the selective determination of arsenic(III) was obtained by discriminating the presence of arsenic(V) in mild hydrochloric acid. The detection limits (3σ) for the online reduction procedure were 0.037 μgL^{-1} for arsenic(III), 0.033 μgL^{-1} for arsenic(V) and the relative standard deviation (RSD) 1.1%, 1.3%, respectively, for 5 μgL^{-1}. For the selective determination of arsenic(III) the detection limit was 0.111 μgL^{-1} and the RSD was 0.7% (n=l0) at 5.0 μgL^{-1}. The method was applied for drinking water analysis.

Vieira *et al.* (2002) proposed a simple and reliable method for the determination of arsenic in sediment and in coal without digestion, based on hydride generation atomic absorption spectrometry. An aliquot was used for hydride generation with sodium borohydride. More than 80% of the arsenic was leached to the aqueous phase. The arsine generated in the reactor was transported into the graphite tube, treated with 0.5 mg of iridium as a permanent modifier, and the arsenic determination was carried out by electrothermal atomic absorption spectrometry. The limits of detection in the samples were 0.54 and 0.7 μgg^{-1} for the coal and sediment samples, respectively, obtained for 1 mL of slurry containing 1 mg of sample. The method was also applied for the analysis of three sediment samples collected in three locations (Pinheira Beach in Palhoça, Sambaqui and Itacorubi in the North Bay) around the Island of Santa Catarina, Brazil. The concentrations measured with the proposed technique are in reasonable agreement with those obtained earlier by inductively coupled plasma mass spectrometry.

MATERIALS AND METHODS

Materials

1. Apparatus

1.1 Voltammograms were recorded with potentiostat PGSTAT20 (Autolab, Netherlands.), interfaced to IME663 (Eco chemie), 663 VA stand (Metrohm, Swizerland.) and Socos computer. The photograph of complete set up is shown in Appendix Figure C1.

1.2 The electrochemical cell, shown in Appendix Figures C2-C3, consisted of a gold electrode (lateral) served as a working electrode, an Ag/AgCl with saturated KCl served as reference electrode and a platinum wire served as an auxiliary electrode (all of them from Metrohm, Swizerland).

1.3 A vapor generation accessory (VGA-77) unit connected to electrothermal temperature controller (ETC-60) and an atomic absorption spectrometer (Spectra AA880Z, Varian, USA). The instrument was shown in Appendix Figure C4.

1.4 The pH was measured using a Corning model 430 pH meter (USA).

1.5 Ultra pure water was prepared using a water purification system, Simplicity^{TM} (Millpore, France)

1.6 An artificial neural network (ANN) software version 5.0 from NeuroDimension, Inc.(Gainesville, Florida, USA).

2. Reagents

All chemicals used were listed in Appendix B. Ultra pure water was used throughout these studies.

2.1 Reagent for preparation of standard solution

- Standard arsenic stock solution 1000 ppm arsenic(III) was prepared by dissolving 1.320 gram of arsenic trioxide (As_2O_3) in a minimum amount of sodium hydroxide (NaOH) solution. The solution was acidified to pH 3.5 with hydrochloric acid (HCl) and diluted to 100 mL with ultra pure water. A 0.3 gram 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 ^oC was stable for at least 1 month. Working solutions of the desired arsenic(III) concentrations were prepared from the stock solution daily.

- Copper(II) standard stock solution 1000 ppm copper(II) was prepared by dissolving 0.393 gram of copper(II)sulfate pentahydrate ($CuSO_4.5H_2O$) in ultra pure water and made up volume to 100 mL with ultra pure water. The required standards were prepared daily by dilution of the stock solutions with ultra pure water.

- Lead(II) standard stock solution 1000 ppm lead(II) was prepared by dissolving 0.160 gram of lead(II)nitrate ($Pb(NO_3)_2$) in ultra pure water and made up volume to 100 mL with ultra pure water. The required standards were prepared daily by dilution of the stock solutions with ultra pure water.

- Water samples were preserved by on-site acidification with HCl to a pH ≤ 2 and filtering the sample ($< 0.45 \mu m$) and keeping the samples in opaque bottles under temperature at 4^oC. Under these conditions, arsenic redox species were stable for more than a year.

2.2 Reagent for anodic stripping voltammetry technique

- The mixing solution of 5 M sulfuric acid(H_2SO_4) and 0.5 M hydrochloric acid(HCl) supporting electrolyte was prepared by mixing the solution between 10.75 mL of conc. HCl and 70 mL of conc.H_2SO_4 and then made up volume to 250 mL with ultra pure water.

- 1 M sulfuric acid solution was prepared by adding 5.6 mL of conc. H_2SO_4 and made up volume to 100 mL with ultra pure water.

- 1% hydrazinium sulfate solution was prepared by dissolving 2.0422 gram of hydrazinium sulfate in ultra pure water and made up volume to 100 mL.

- 0.1 M nitric acid solution was prepared by adding 0.63 mL of conc. HNO_3 and made up volume to 100 mL with ultra pure water.

2.3 Reagents for hydride generation atomic absorption spectrometry technique

- 0.6 M $NaBH_4$ solution was freshly prepared by dissolving 1.5 gram of $NaBH_4$ in 250 mL of 0.5 % NaOH.

- 5 M HCl solution was prepared by adding 125 mL of conc HCl and made up volume to 250 mL with ultra pure water.

Methods

Anodic Stripping Voltammetric Method

1. Pretreatment of the gold electrode

Prior to use, the gold electrode was polished by a fine sand paper (1200CW) and finally polished with alumina powder. After polishing, the electrode should be immersed into 0.1 M HNO_3 for 2-3 minutes to dissolve the remaining Al_2O_3 particles from the electrode surface and washed it successively with distilled water. The electrochemical activation of the electrode surface was realized by cyclic voltammetry, with solution of 1 M H_2SO_4 as supporting electrolyte-saturated nitrogen (15 min). The electrode was polarized between the potential values of 0.20 and 1.90 V using a scan rate 500 mVs^{-1} and 50 cycles were performed. After approximate 20 cycles, the course of the measured voltammetric curves became stable and remained unchanged during further cycles. This phenomenon serves as verification of activation of the electrode surface. The electrode could be used only one measurement, the electrochemical activation of the surface should be repeated before next measurement without polishing. The cyclic voltammogram of activation of gold electrode was shown in Figure 6.

2. Determination of arsenic(III)

2.1 Calibration curve and limit of detection of ASV technique

Arsenic(III) standard solutions of 0.25, 0.5, 1, 2.5, 5, 10, 20, 30, 40, 50, 60, 80 and 100 ppb in ultra pure water were prepared. The gold electrode, Ag/AgCl reference electrode and platinum wire counter electrode were placed in electrochemical cell. Ten mL of arsenic(III) standard solution and 2.5 mL of 5 M H_2SO_4 and 0.5 M HCl supporting electrolyte were pipetted in the electrochemical cell. Purged the solution with nitrogen for 10 minutes. The ASV was applied for measurement using the conditions in Table 2 and the voltammograms were recorded. Triplicate determinations were performed on each standard solution. The calibration data were plotted between the peak area against arsenic(III) concentration. The voltammograms and calibration curve were presented in Figures 7 and 8. The linear dynamic range was established from a ten point calibration curve (ie., 0.25, 0.5, 1, 2.5. 5, 10, 20, 30, 40 and 50 ppb of arsenic(III)) and limit of detection (LOD) was also determined.

Table 2 Conditions of calibration curve of ASV method for determination of arsenic(III)

Parameters	**Description**
Conditioning potential	0.5 V for 60 s
Deposition potential	-0.2 V for 180 s
Equilibration time	30 s
Initial potential	-0.2 V
End potential	0.7 V
Scan rate	80 mVs^{-1}
Modulation amplitude	0.1 V

2.2 Precision study

The precision was studied by determination of arsenic(III) concentration at 5 ppb and measurement 10 replicates using the same conditions as calibration method. The results were presented in Table 10.

2.3 Interference study

The interfering ions used in the study of effect of interferences in determination of arsenic(III) at 5 ppb were arsenic(V), iron(II), cadmium(II), copper(II) and lead(II). In the studies, concentration of interference ions against to arsenic(III) used in the studies were shown in Table 3 . The ASV analysis was carried out using the same conditions as calibration method. The results of each interference ion study were presented in Tables 11-15.

Table 3 Concentration of interfering ions against to arsenic(III) used in the study of effect of interferences in determination of arsenic(III)

Interfering ions / arsenic(III)	**Concentration of interfering ion / arsenic (molar fold excess)**
As(V)	0, 200, 400, 600, 800, 1000
Fe(II)	0, 50, 100, 200, 800, 1500, 2000, 3000
Cd(II)	0, 10, 20, 40, 80, 100, 120
Pb(II)	0, 1, 5, 10, 15, 20
Cu(II)	0, 1, 2, 3, 4, 5

2.4 Interaction study

The response of measurement system depends on a variety of experimental factors (ie., concentration of interfering ions). To know which factors are main factors, factorial design was performed at two levels i.e. low and high. In the studies, the factors were concentration of arsenic(III), copper(II), and lead(II). This means that there are 8 possible combinations of factor levels, as shown in Table 4. A plus sign denotes that the factor is at the high level and a minus sign that is at the low level. The high level of concentration of arsenic(III), copper(II), and lead(II) are 50, 15, and 100 ppb, respectively. The low level of concentration of arsenic(III), copper(II), and lead(II) are 2.5, 0, and 30 ppb, respectively. The number 1 is used to indicate that all factors are at the low level. The ASV analysis was carried out using the same conditions as described in the calibration method. The results using the two-way ANOVA calculations with interaction were presented in Table 16.

Table 4 Factorial experimental design used in the study of effects of the factors and interactions in the determination of arsenic(III)

Combination of factor levels	**As(III)**	**Cu(II)**	**Pb(II)**
1	-	-	-
As	+	-	-
Cu	-	+	-
Pb	-	-	+
Cu Pb	-	+	+
As Pb	+	-	+
As Cu	+	+	-
As Cu Pb	+	+	+

2.5 Elimination of interfering ions

Ten ml of copper(II) standard solution and supporting electrolyte solution containing 5 M sulfuric acid, 0.5 M hydrochloric acid and $1.0\text{x}10^{-5}$ M cupferron were pipetted into the electrochemical cell and the solution was purged with nitrogen gas for at least 10 min. The ASV was applied for measurement using the conditions in Table 2 and the voltammograms were recorded. The results were presented in Table 17.

Twenty five milliliters of an aqueous standard solution containing mixture solution of arsenic(III), copper(II) and lead(II) ions were placed in a separatory funnel. Ammonium-pyrrolidindithiocarbamate (APDC) solution was added and the sample solution was mixed thoroughly. Duplicate extractions were performed with 10 mL dichloromethane for each extraction. The resultant aqueous layer was analyzed by ASV technique using the same conditions as calibration method. The results were presented in Table 18.

2.6 An artificial neural networks (ANN)

Ten mL of standard mixture solution of arsenic(III), copper(II) and lead(II) was pipetted into the electrochemical cell and 2.5 ml of 0.5 M hydrochloric acid and 5 M sulfuric acid served as supporting electrolyte were added to the solution. After the solution was deaerated with N_2 gas (OFN) for 10 minutes. The deposition potential, -0.2 V, was applied to the gold electrode for 3 min while the solution was stirred. After deposition step, the magnetic stirrer was turn off and left for 30 seconds at -0.2 V so that the solution became quiescent. The stripping was carried out in the differential pulse mode starting at -0.2 V to 0.7 V with scan rate and modulation amplitude of 80 mVs^{-1} and 0.1 V, respectively. The currents of 30 points of voltage were recorded for each solution. The data of 50 voltammograms were inputs for the artificial neural network.

2.6.1 Optimization of ANN method for determination of arsenic(III)

The artificial neural network, which builds in NeuralExpert program used Function Approximation for building the best network and configures the parameters. The results from ASV technique were divided into two set, training set and test set, as input for training neural network. The neural network performed the functional relationship between the input and desired outputs.

2.6.1.1 Transfer functions and numbers of hidden layers study

In the study of transfer functions both sigmoid and tanh functions were used to the output of summing function into a real output via corresponding algorithm. The training 5 times using the condition presented in Tables 5 and 6. The type of transfer functions and numbers of hidden layers were investigated. The networks were shown in Figures 2 and 5 and the results were demonstrated in Tables 19 and 20.

Table 5 Condition for Transfer functions study

Parameters	**Description**
Transfer function	Sigmoid, tanh
Hidden layer	One
Processing element	8
Learning rate	0.5
Momentum rate	0.5
Epochs	5000

Table 6 Condition for hidden layer study

Parameters	**Description**
Transfer function	Sigmoid, tanh
Hidden layer	Two
Processing element	8
Learning rate	0.5
Momentum rate	0.5
Epochs	5000

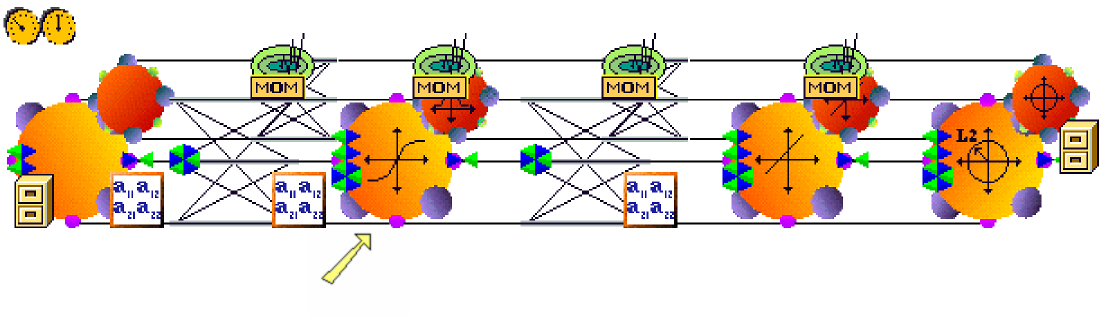

Figure 2 The ANN using tanh transfer function for one hidden layer
(network 1)

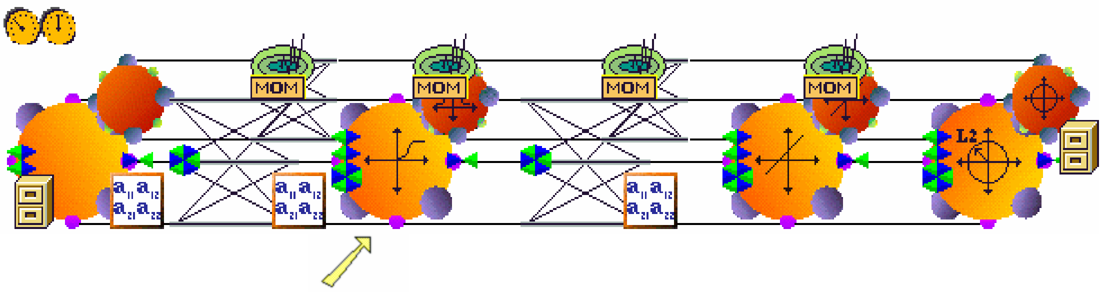

Figure 3 The ANN using sigmoid transfer function for one hidden layer
(network 2)

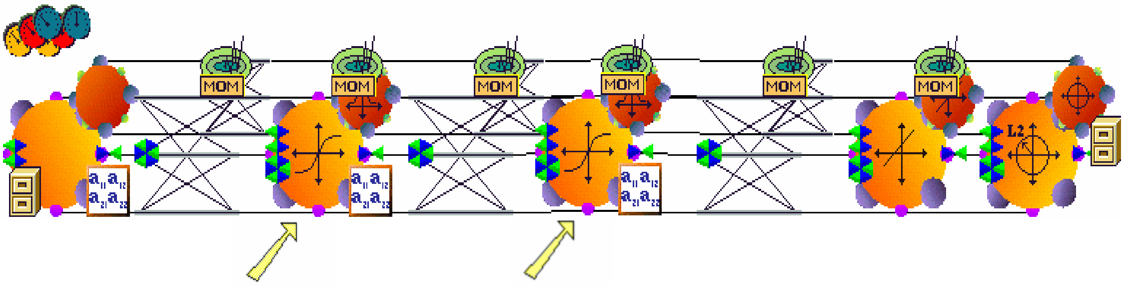

Figure 4 The ANN using tanh transfer function for two hidden layers
(network 3)

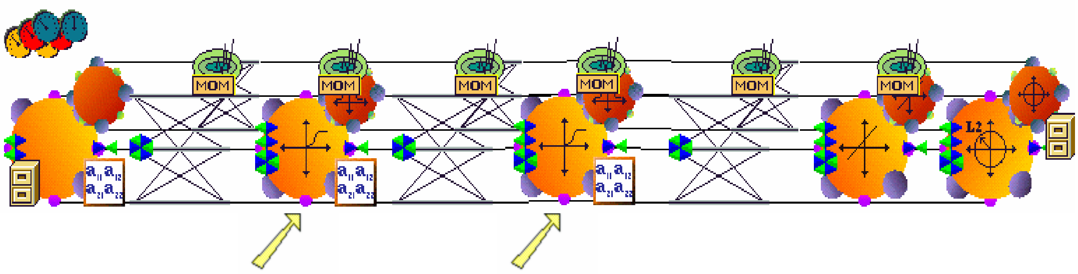

Figure 5 The ANN using sigmoid transfer function for two hidden layers
(network 4)

2.6.1.2 Study of number of processing elements (PE)

The number of processing element was achieved by following the same condition as study of transfer function and using tanh function for two hidden layers. The processing element used in study was shown in Table 7. The results were presented in Tables 21 and 22 and the results were plotted between processing elements (PE) and mean square error (MSE) as shown in Figures 9 and 10.

Table 7 Condition for number of processing elements study

Varied PE for hidden one (8 PE for hidden two)	**Varied PE for hidden two (6 PE for hidden two)**
2	2
4	4
6	6
8	8
10	10
12	12

2.6.1.3 Learning rate study

The network was trained with the same conditions as described in the study of a number of processing elements and using 6 processing elements for hidden one and hidden two. Learning rate varied was 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9. The results were presented in Table 23 and the results were plotted between learning rate and mean square error (MSE) as shown in Figure 11.

2.6.1.4 Momentum rate study

The network was trained with the same conditions as described in the study of leaning rate and using 0.4 learning rate. Momentum rate varied was 0.1, 0.2, 0.25, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9. The results were presented in Table 24 and the results were plotted between momentum rate and mean square error (MSE) as shown in Figure 12.

2.6.1.5 Number of epochs study

In order to optimize the number of epochs in the network, ANN architectures with different number of epochs were examined with 100, 500, 1,000, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000 and 10,000 at the optimized condition. The results were presented in Table 25 and the results were plotted between numbers of epochs and mean square error (MSE) as shown in Figures 13 and15.

2.7 Determination of arsenic(III) in ground water samples from Ron Piboon District using ASV technique and data processing by artificial neural networks

Ten ml of water sample solution and 2.5 ml of 0.5 M HCl and 5 M H_2SO_4 served as supporting electrolyte were transferred to the electrochemical cell and then the solution was deaerated with N_2 gas (OFN) for 10 minutes. The ASV analysis was achieved by following the condition as described in calibration method. The characteristics of the optimized networks were used to predict the arsenic(III) content in the samples. The optimized conditions of an artificial neural network were present in Table 8. The results of ground water samples observed by ASV technique were shown in Table 26.

Table 8 The optimized conditions of an artificial neural network

Parameters	**Description**
Transfer function	tanh
Hidden layer	two layer
Processing element of hidden one	6
Processing element of hidden two	6
Learning rate	0.4
Momentum rate	0.25
Epochs	8000

Hydride Generation Atomic Absorption Spectrometric Method

Determination of arsenic(III) was carried out using HG-AAS. In the system, arsenic containing in samples, 0.6% $NaBH_4$ solution, 5 M HCl were pumped from their containers using a peristaltic pump. The flow rates were used 7 $mLmin^{-1}$ for sample and 1 $mLmin^{-1}$ for reagents. Three solutions left to react in the 1 m long mixing coil. The inlet side of the mixing coil was supplied by nitrogen flow gas to carry out the generated arsine(AsH_3). After arsine(AsH_3) was separated from the solution at the gas-liquid separator and was swept out by nitrogen flow gas to the T-shape quartz tube as an absorption cell. Arsine was atomized at temperature of 925 ^oC by the temperature controller system. Finally, the atomized arsine was detected. The Operating conditions of HG-AAS were shown in Table 9. Triplicate determinations were performed on each standard solution. The calibration curve was presented in Figure 16. The results of determination of arsenic(III) in ground water samples from Ron Piboon District by HG-AAS technique were shown in Table 27.

Table 9 Operating conditions of HG-AAS system

Instrument modes	**Descriptions**
Atomizer	T-shape quartz tube
Lamp	Hallow cathode lamp
Vapor type	Electric hydride
Reducing agent	0.6 % $NaBH_4$ in 0.5% NaOH
Acid solution	5 M HCl solution
Carrier gas	Nitrogen gas (99.99%)
Wavelength	193.7 nm
Slit width	1.0 nm
Lamp current	12 mA
Calibration	Concentration
Measurement	Integrate
Replicates (standard / sample)	3
Measurement time	7.0 s

RESULTS AND DISCUSSIONS

Results Obtained from Anodic Stripping Voltammetric Technique

1. Pretreatment of the gold electrode

Before each analysis, the gold electrode was well cleaned with 1 M H_2SO_4. The cyclic voltammogram of activation of the gold electrode was shown in Figure 6.

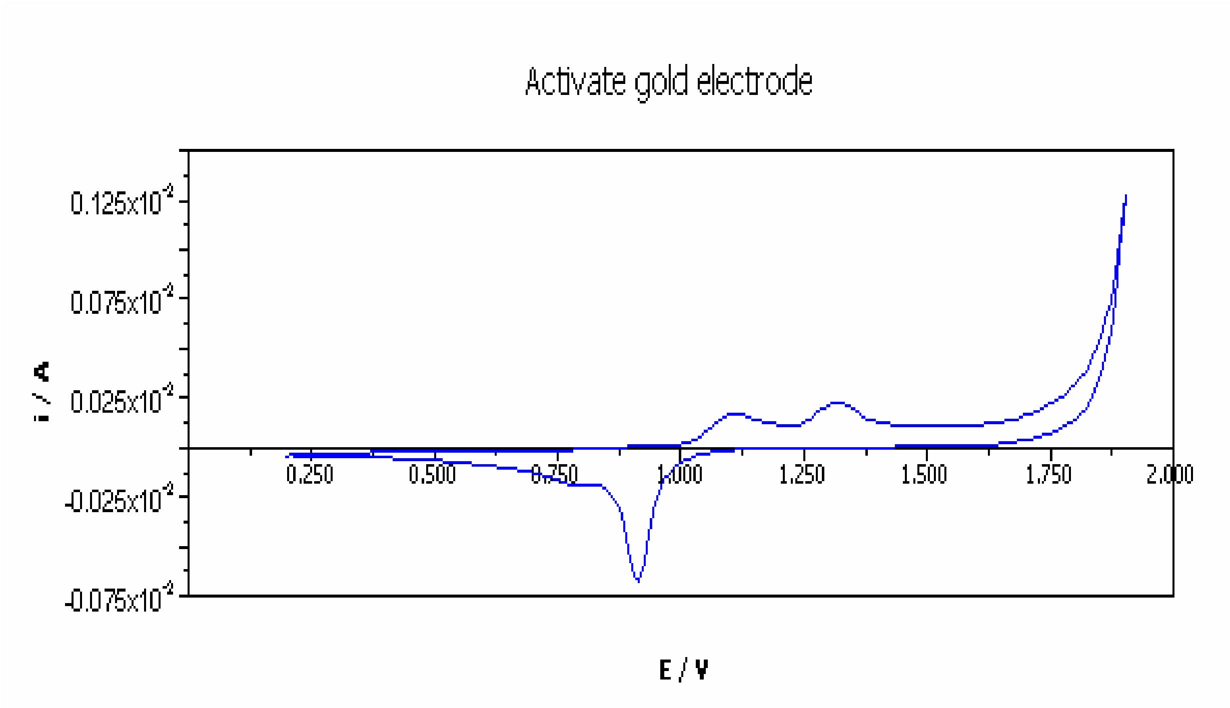

Figure 6 Cyclic voltammogram of the activation of the gold electrode

2. Determination of arsenic(III)

2.1 Calibration curve and limit of detection of ASV technique

The linearity between the arsenic(III) concentration and the anodic peak current was obtained in a wide concentration range (between 0.25 and 50 ppb), when 180 s electrolysis time was applied. In a higher concentration range of 60-100 ppb, the calibration already exhibited a typical curvature due to saturation of the electrode surface with the analyte at high concentration. The sensitivity of this method shown as the slope of the calibration line was $4.705x10^{-8}$AV/ppb and the correlation coefficient of the corresponding relationship was 0.9998 in the range of 0.25 to 50 ppb arsenic(III). The voltammogram and calibration curve were shown in Figures 7 and 8.

From the calibration curve of arsenic(III), the limit of detection (LOD) was determined with the value of 0.25 ppb (Miller, 2000) which was lower than the maximum contaminant level (MCL) of EPA permit for dissolved arsenic in drinking water (10 ppb).

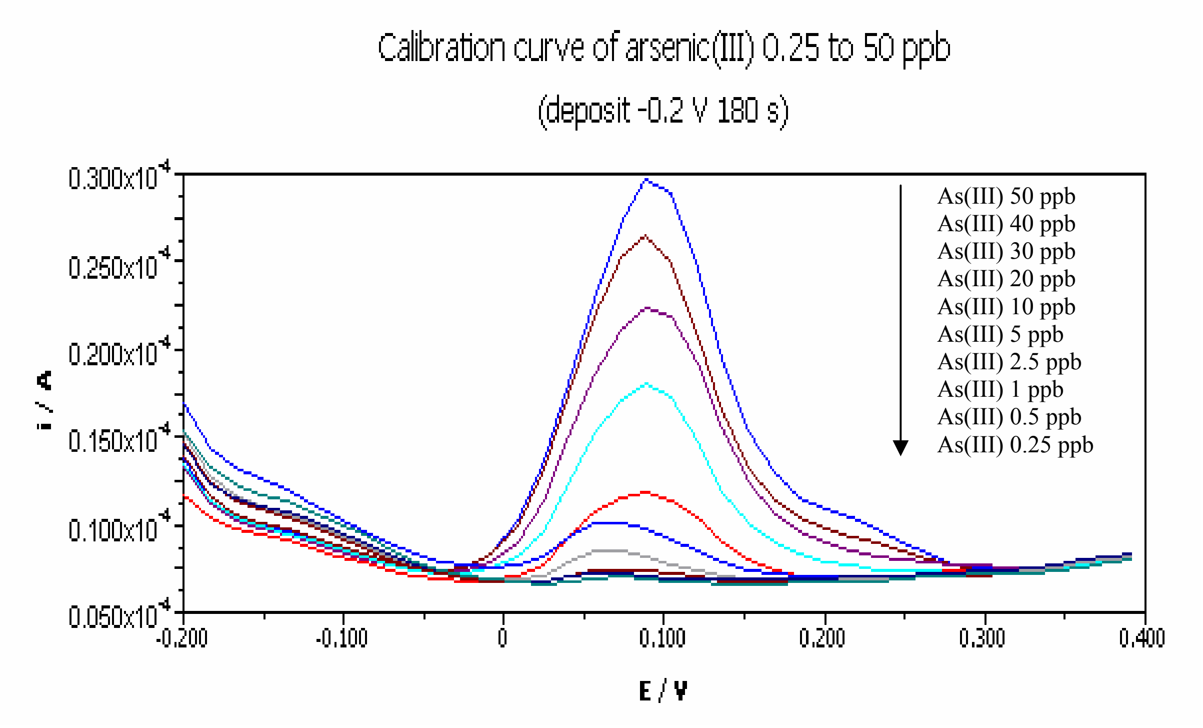

Figure 7 Voltammograms of arsenic(III) obtained from ASV technique

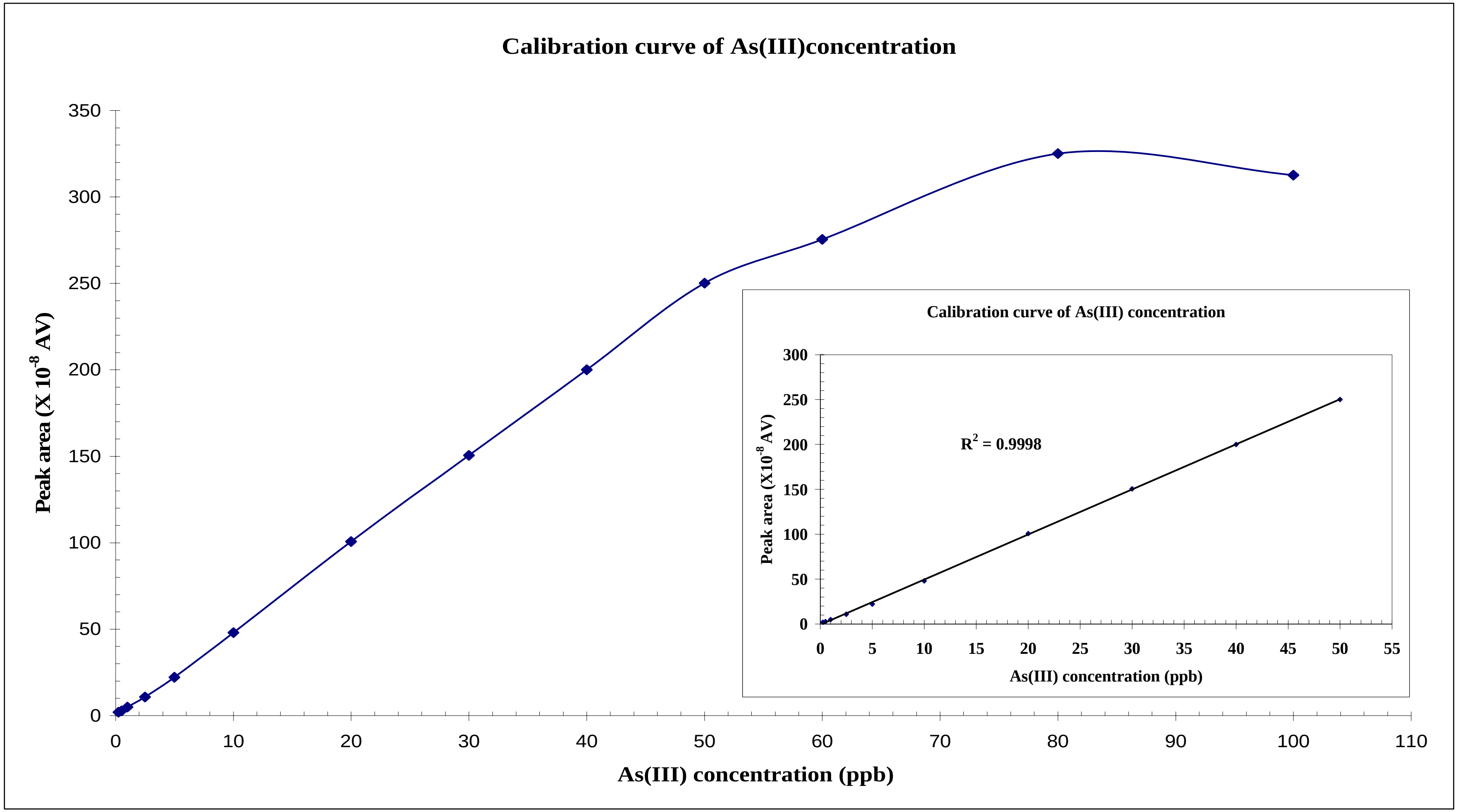

Figure 8 Calibration curve for arsenic(III) obtained from ASV technique

2.2 Precision study

The precision was studied at 5 ppb arsenic(III) and measurement 10 replicates. The results were presented in the Table 10 and the relative standard deviation (R.S.D.) was calculated to be 3 %.

Table 10 The precision of ASV technique

Replicate No.	**Peak area (x10^{-7} AV)**
1	2.047
2	2.021
3	2.025
4	2.109
5	1.926
6	1.946
7	1.949
8	1.967
9	2.076
10	2.000
Average peak area ($\overline{X}$)	2.007
Standard deviation (SD.)	0.060
R.S.D%	2.998

R.S.D. = The relative standard deviation

2.3 Interferences study

Interference caused by common substances present in well water, i.e. arsenic(V), copper(II), lead(II), iron(II) and cadmium(II) were investigated. The ASV response to an aqueous solution containing 5 ppb arsenic(III) and different levels of interfering ions were shown in Tables 11 and 15.

For determination of arsenic(III) at 5 ppb, it was found that 3,000-fold of iron(II), 1,000-fold of arsenic(V) did not interfere and in the presence of 100-fold of cadmium(II) did not effect in the determination of arsenic(III). If amount of interferences were greater than these values, they gave the negative error for determination of arsenic(III).

The most serious interferences for electro-analytical measurement of arsenic(III) at a gold electrode were copper(II) and lead(II). If it had only 1-fold of these metals present in the solution, it gave the negative error result.

Table 11 Effect of arsenic(V) on the determination of arsenic(III) at 5 ppb

Arsenic(V) / Arsenic(III) (mole ratio)	**Peak area of arsenic(III) ($\times10^{-7}$ AV)**
0	3.012
200	2.941
400	3.065
600	2.906
800	2.911
1000	3.077

Table 12 Effect of iron(II) on the determination of arsenic(III) at 5 ppb

Iron(II) / Arsenic(III) (mole ratio)	**Peak area of arsenic(III) ($x10^{-7}$ AV)**
0	2.267
50	2.305
100	2.369
200	2.348
800	2.318
1500	2.300
2000	2.349
3000	2.246

Table 13 Effect of cadmium(II) on the determination of arsenic(III) at 5 ppb

Cadmium (II) / Arsenic(III) (mole ratio)	**Peak area of arsenic(III) ($x10^{-7}$ AV)**
0	2.246
10	2.171
20	2.314
40	2.348
80	2.311
100	1.974
120	1.875

Table 14 Effect of lead(II) on the determination of arsenic(III) at 5 ppb

Lead(II) / Arsenic(III) (mole ratio)	**Peak area of arsenic(III) ($x10^{-7}$ AV)**
0	2.190
1	1.995
5	0.998
10	0.299
15	0.264
20	0.210

Table 15 Effect of copper(II) on the determination of arsenic(III) at 5 ppb

Copper(II) / Arsenic(III) (mole ratio)	**Peak area of arsenic(III) ($x10^{-7}$ AV)**
0	2.227
1	1.826
2	1.695
3	1.569
4	1.200
5	0.898

2.4 Interactions study

From interference study, the most serious interfering ions for electro-analytical measurement of arsenic(III) at a gold electrode are copper(II) and lead(II). The results were shown in Table 16 and the interactions between two factor, arsenic(III) and copper(II), arsenic(III) and lead(II), were calculated to be $1.871\text{x}10^{-7}$ AV and $7.922\text{x}10^{-8}$ AV, respectively. To test for significance of copper(II) and lead(II) effect. ANOVA was used and found that interaction between all three factors is significant. This means that copper(II) and lead(II) interact strongly with arsenic(III).

Table 16 Interactions study between two factors, copper(II) and lead(II) on the determination of arsenic(III) at two levels.

Combination of factor levels	**As(III)**	**Cu(II)**	**Pb(II)**	**Area (AV)**	
				Replicate 1	**Replicate 2**
1	-	-	-	$8.146\text{x}10^{-8}$	$8.063\text{x}10^{-8}$
As	+	-	-	$2.214\text{x}10^{-6}$	$2.130\text{x}10^{-6}$
Cu	-	+	-	$6.224\text{x}10^{-8}$	$6.449\text{x}10^{-8}$
Pb	-	-	+	$7.060\text{x}10^{-8}$	$7.032\text{x}10^{-8}$
Cu Pb	-	+	+	$6.403\text{x}10^{-8}$	$6.623\text{x}10^{-8}$
As Pb	+	-	+	$2.063\text{x}10^{-6}$	$2.089\text{x}10^{-6}$
As Cu	+	+	-	$1.886\text{x}10^{-6}$	$1.839\text{x}10^{-6}$
As Cu Pb	+	+	+	$1.672\text{x}10^{-6}$	$1.691\text{x}10^{-6}$

2.5 Elimination of interfering ions

To eliminate interference from copper(II) ion, cupferron was added to complex copper but under optimized condition for arsenic(III), both the position and area of copper(II) were the same as shown in Table 17.

The next attempt, therefore, was to use ammonium-pyrrolidin dithiocarbamate (APDC) to coprecipitate copper(II) and lead(II) as M-PDC complex, Again there is no success as arsenic(III) also coprecipitated, leading to the decrease in peak area as shown in Table 18.

Table 17 Elimination of copper(II) ion with cupferron at copper(II) 10 ppb

Copper(II) / Cupferron (mole ratio)	Peak area of copper(II) ($x10^{-8}$ AV)
0	7.470
50	7.509
100	7.481

Table 18 Elimination of interfering ions with APDC at arsenic(III) 50 ppb

Mixture solutions	Peak area ($x10^{-7}$AV)		
	Arsenic(III)	Copper(II)	Lead(II)
Before extraction with APDC	1.425	1.637	3.855
After extraction with APDC	no peak	no peak	2.739

2.6 An artificial neural networks (ANN)

2.6.1 Optimization of ANN method for determination of arsenic(III)

The network parameters that should be optimized are transfer functions in the layers, number of layers, momentum, learning rate and number of epochs. The successful network should have a constantly decreasing slope of the training mean square error (MSE) to minimum value.

2.6.1.1 Transfer functions and numbers of hidden layers study

The most versatile transfer functions that can be used to model a variety of non-linear relationships are a sigmoid and tanh transfer functions. Four types of ANN structures are established to evaluate the learning performance of the training network. Table 19 shows mean square error (MSE) values obtained from one hidden layer with sigmoid and tanh. From the results, the sigmoid transfer function gave a value of MSE lower than tanh transfer function for one hidden layer. Table 20 shows MSE values obtained from two hidden layer with sigmoid and tanh. The tanh transfer function gave a value of MSE lower than sigmoid function for two hidden layers. Compared network 3 (Table 20) to network 2 (Table 19), the MSE of network 3 with tanh functions is ten fold lower than that of network 2.

Table 19 Transfer function study between sigmoid and tanh for one hidden layer

Replicate No.	**Mean square error (MSE)**	
	Network 1 (tanh)	**Network 2 (sigmoid)**
1	0.0267	$1.843x10^{-5}$
2	$1.07x10^{-5}$	$3.905x10^{-5}$
3	0.2671	$2.232x10^{-5}$
4	0.0047	$2.928x10^{-5}$
5	0.0267	$7.368x10^{-6}$
Average	0.0651	$2.329x10^{-5}$

Table 20 Transfer function study between sigmoid and tanh for two hidden layers

Replicate No.	**Mean square error (MSE)**	
	Network 3 (tanh)	**Network 4 (sigmoid)**
1	$2.030\text{x}10^{-7}$	0.0224
2	$2.471\text{x}10^{-6}$	0.0228
3	$2.684\text{x}10^{-6}$	0.0227
4	$2.796\text{x}10^{-6}$	0.0224
5	$2.957\text{x}10^{-6}$	0.0228
Average	$2.222\text{x}10^{-6}$	0.0226

2.6.1.2 Number of processing elements (PE) study

The numbers of processing element in the input, output and hidden layers depend on the nature of problem to be solved. If the numbers of hidden neurons is too small, the network may not have performance to learn the process correctly. If the number is too high, the training will take a long time and the network may overfit the data. The ANNs are trained with a training set of input and known output data for optimization processing element (PE) at two hidden layers. From the results, the optimum processing elements were 6 for both hidden one and hidden two layers. The results were presented in Tables 21 and 22 and the results plotted between processing element (PE) and mean square error (MSE) were shown in Figures 9 and 10.

Table 21 Number of processing elements (PE) study for hidden one

Processing elements No.		Mean square error ($x10^{-6}$)			
Hidden one	**Hidden two**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**
2	8	2.511	3.118	7.069	4.232
4	8	1.018	1.743	3.432	2.064
6	8	2.495	1.650	1.814	1.986
8	8	2.510	3.009	1.735	2.418
10	8	2.455	6.226	4.665	4.449
12	8	3.150	2.963	3.277	3.130

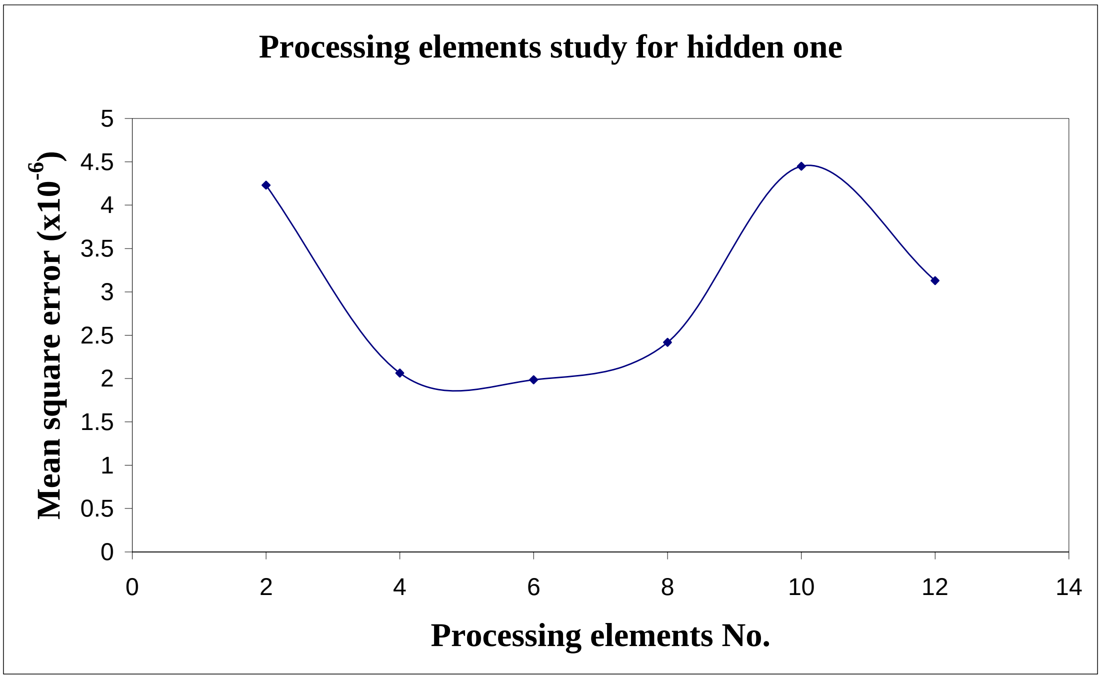

Figure 9 Number of processing elements (PE) study for hidden one

Table 22 Number of processing elements (PE) study for hidden two

Processing elements No.		**Mean square error ($x10^{-6}$)**			
Hidden one	**Hidden two**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**
6	2	2.783	3.237	4.462	3.494
6	4	3.452	1.754	1.544	2.250
6	6	1.911	2.823	1.494	2.076
6	8	1.519	2.523	3.069	2.371
6	10	2.839	6.542	4.780	4.721
6	12	5.381	1.032	1.616	5.772

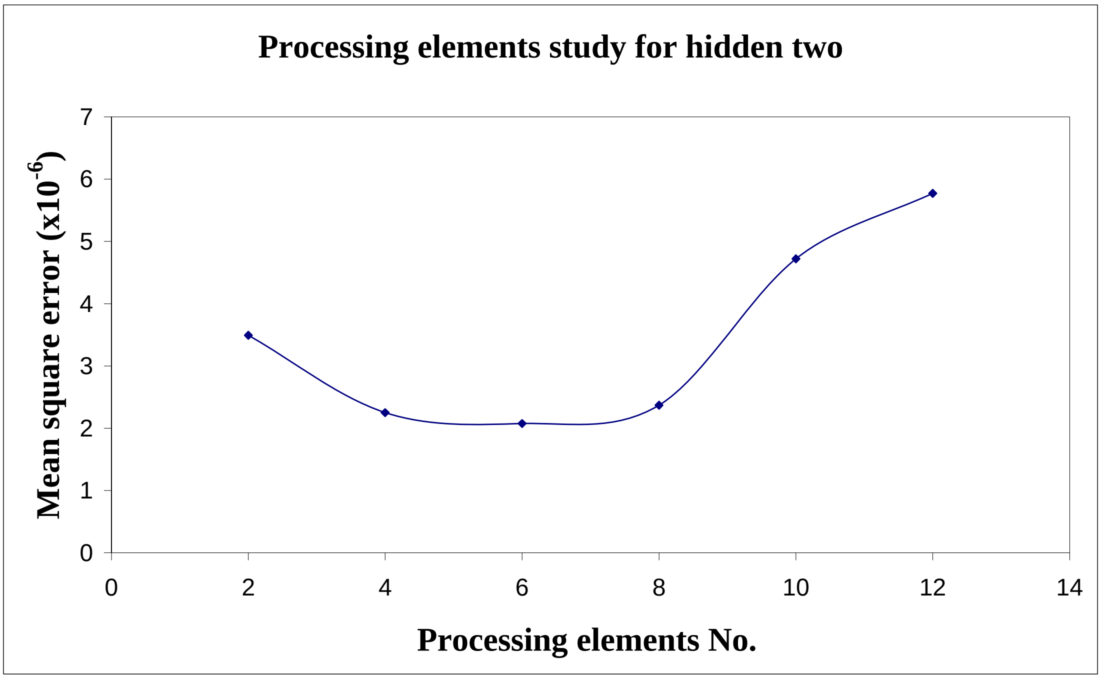

Figure 10 Numbers of processing elements (PE) study for hidden two

2.6.1.3 Learning rate study

Learning rate has value between zero and one. If the learning rate is greater than one, it is easy for the learning algorithm to overshoot in correcting the weights, and the network will oscillate. Small values of the learning rate will not correct the current error as quickly but if small steps are take in correct errors. For these reasons, the effect of the number of learning rates in the network performance was considered. The results of learning rates study and the plot of the number of learning rate against mean square error (MSE) were shown in Table 23 and Figure 11, respectively. The learning rate of 0.4 gave minimum MSE and was selected to further studies.

Table 23 Learning rate study

Learning rate	**Mean square error (x10^{-6})**			
	Replicate 1	**Replicate 2**	**Replicate 3**	**Average**
0.1	2.053	5.002	7.075	4.710
0.2	4.503	3.372	3.372	3.749
0.3	4.720	3.058	3.401	3.726
0.4	2.200	1.357	1.410	1.656
0.5	2.823	2.422	4.406	3.217
0.6	2.946	3.679	1.403	2.676
0.7	7.546	4.604	5.714	5.955
0.8	3.658	5.301	1.185	3.384
0.9	4.565	3.017	3.174	3.586

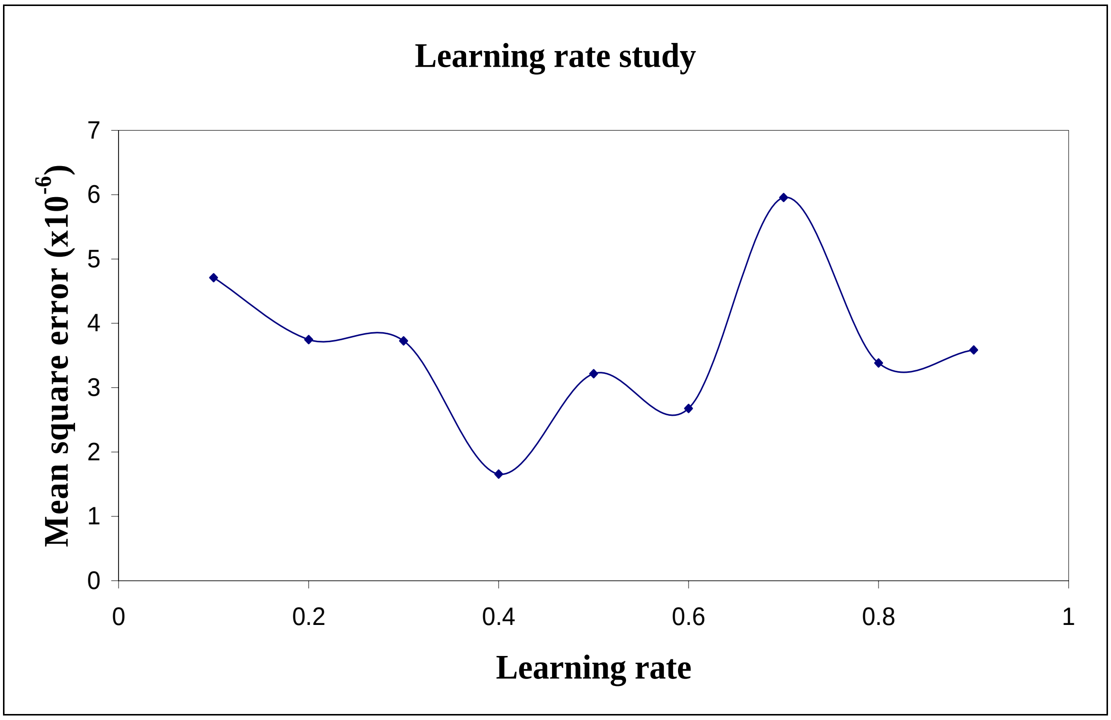

Figure 11 Learning rate study

2.6.1.4 Momentum rate study

Since the neural network is a non-linear optimization system, there is a possibility that the procedure may be ended in a local minimum, if the momentum constant is not selected very carefully. Therefore, momentum values were optimized. Table 24 was showed the results of momentum rate study and the number of momentum rates was plotted against mean square error (MSE) as shown in Figure 12. The momentum rate of 0.25 was chosen because this rate gave the minimum value of mean square error.

Table 24 Momentum rate study

Momentum rate	**Mean square error (x10^{-6})**			
	Replicate 1	**Replicate 2**	**Replicate 3**	**Average**
0.1	4.132	2.590	4.770	3.831
0.2	1.335	4.277	2.117	2.576
0.25	2.554	1.867	1.954	2.125
0.3	1.916	2.729	3.632	2.759
0.4	3.309	5.071	4.127	4.169
0.5	2.526	3.206	6.664	4.132
0.6	3.155	2.239	4.626	3.340
0.7	3.755	1.429	2.511	2.565
0.8	4.904	2.671	4.025	3.867
0.9	2.296	2.324	3.586	2.735

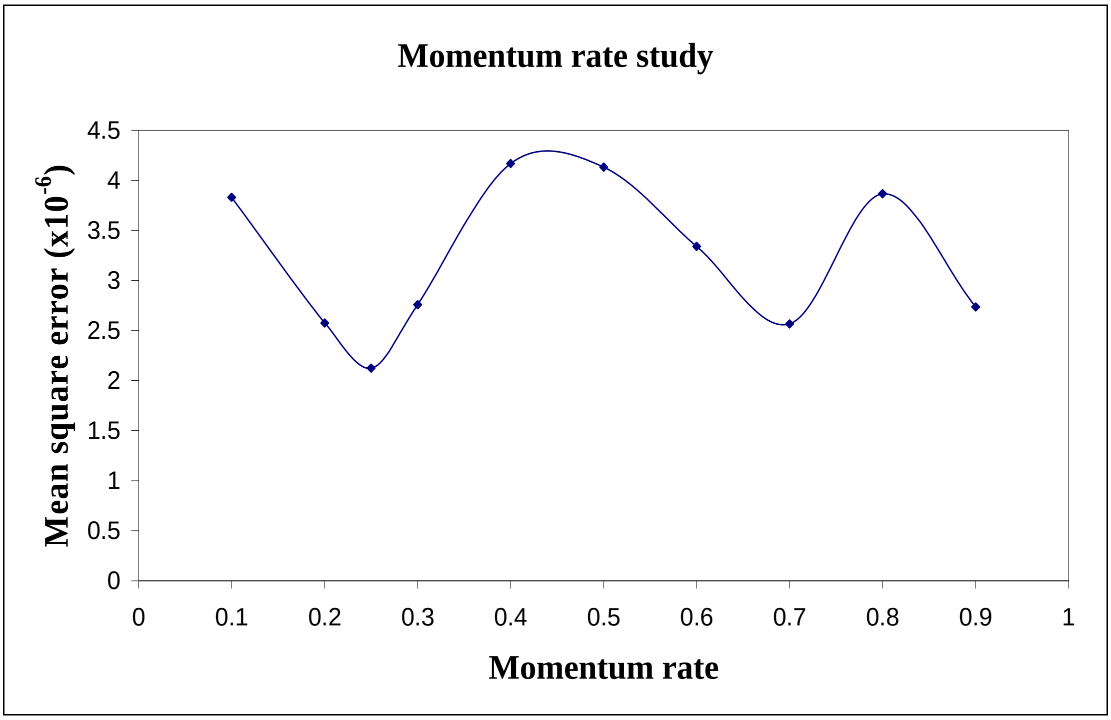

Figure 12 Momentum rate study

2.6.1.5 Number of epochs study

A weakness of the neural network is that it can be easily overfitted, causing the error rate on validation data to be much larger than the error rate on the training data. It is therefore important not to overtrain the data. A good method for choosing the number of training epochs is to use the validation data set periodically to compute the error rate for it while the network is being trained. The validation error decreases in the early epochs of network but after that it begins to increase. The point of minimum validation error is a good indicator of the best number of epochs for training and the weights at that stage are likely to provide the best error rate in new data. The best number of epochs was 8,000. The result of number of epochs study and the plot of the number of epochs against mean square error (MSE) were shown in Table 25 and Figures 13 and 15.

Table 25 Number of epochs study

Number of epochs	**Mean square error ($\times 10^{-6}$)**			
	Replicate 1	**Replicate 2**	**Replicate 3**	**Average**
100	566.24	690.28	327.67	528.07
500	123.03	217.96	201.61	180.87
1000	126.97	161.79	202.81	163.86
2000	124.64	111.81	63.60	100.02
3000	12.14	61.12	17.33	30.20
4000	6.58	4.79	4.25	5.21
5000	2.66	2.19	2.04	2.30
6000	2.53	2.56	1.38	2.16
7000	2.20	2.13	1.07	1.80
8000	1.25	1.88	1.71	1.61
9000	2.83	2.08	1.92	2.28
10000	4.27	3.12	2.89	3.43

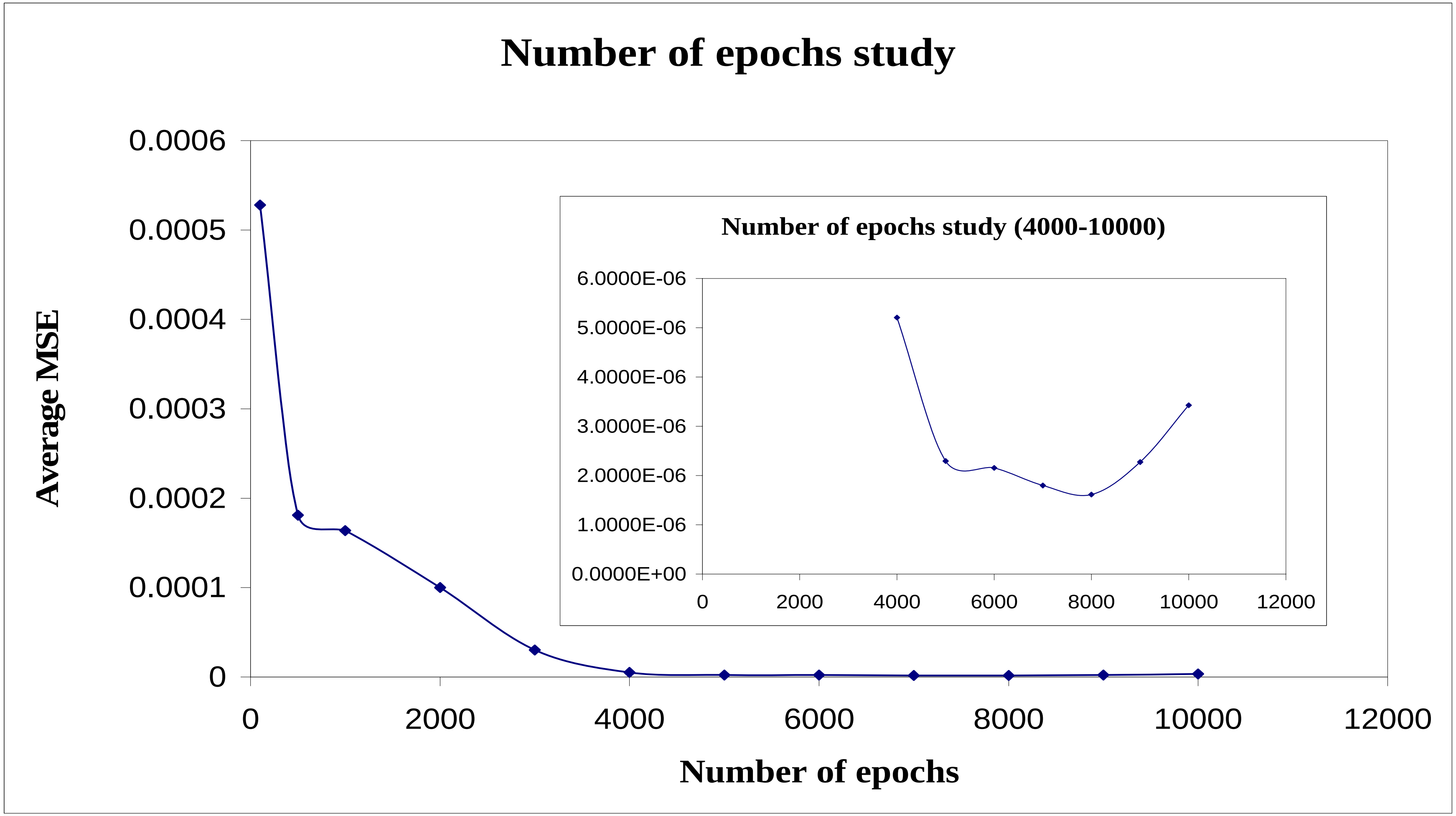

Figure 13 Effect of number of epochs on average mean square error of inputs data

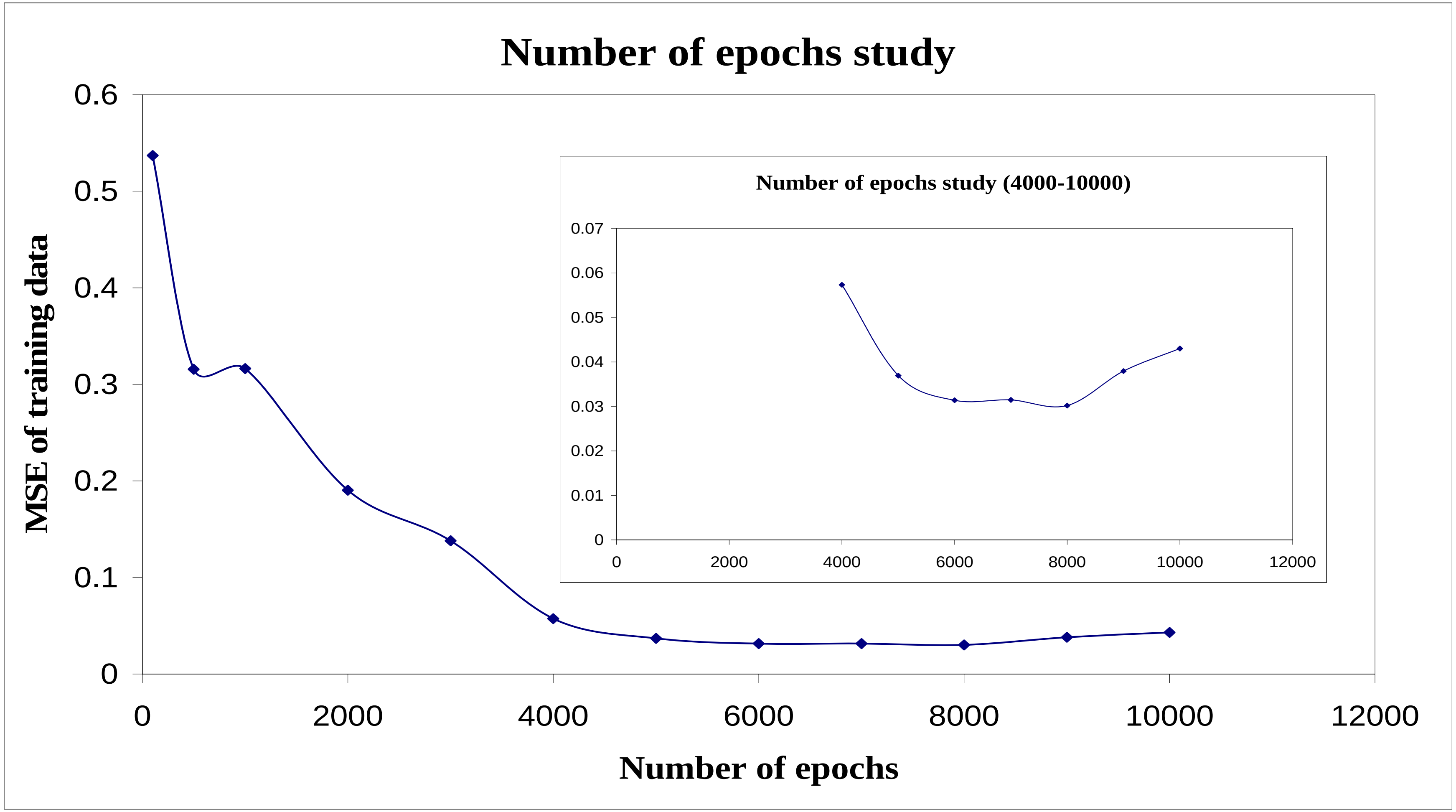

Figure 14 Effect of number of epochs on average mean square error of training data

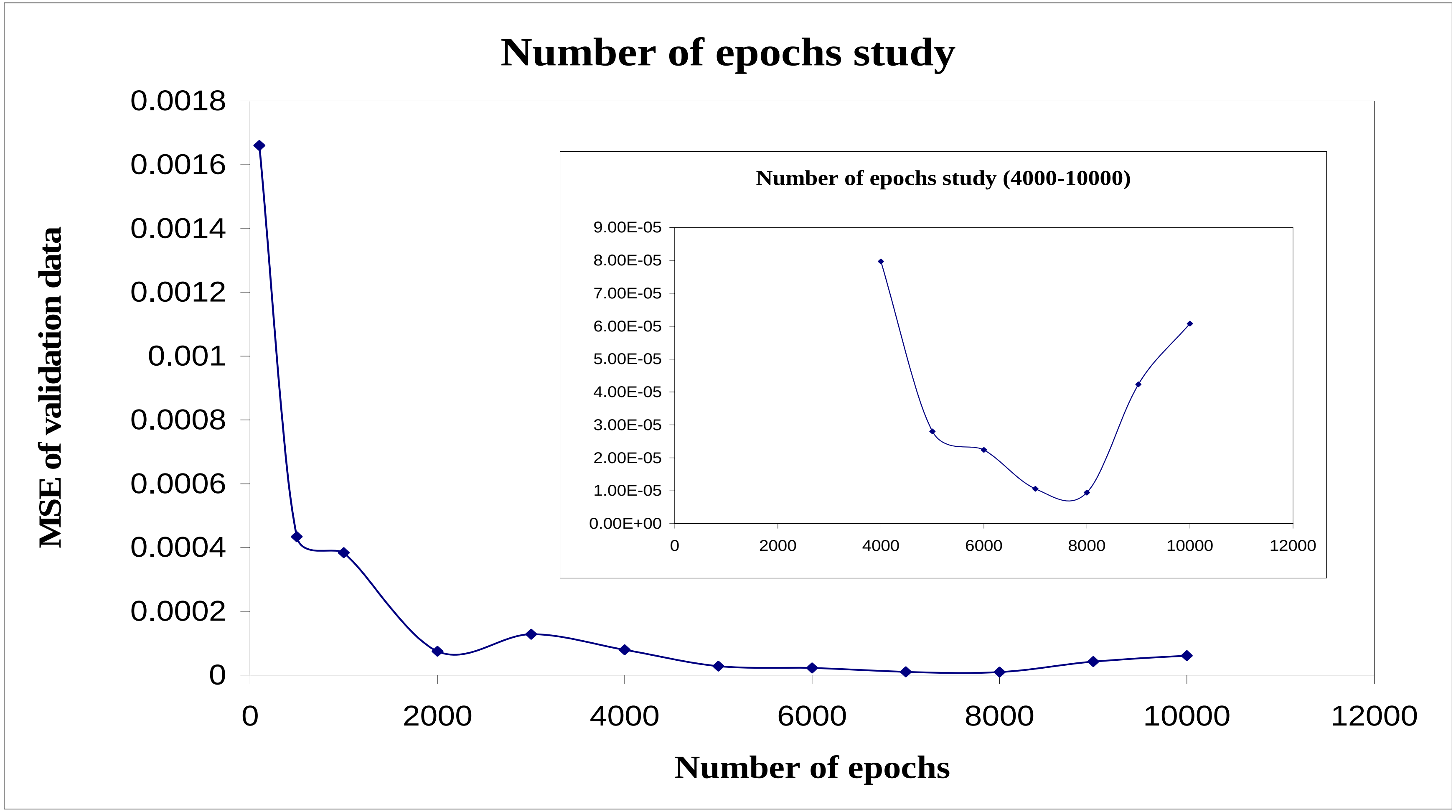

Figure 15 Effect of number of epochs on average mean square error of validation data

The optimized conditions of an artificial neural network were presented in Table 26.

Table 26 The optimized conditions of an artificial neural network

Parameters	**Description**
Transfer function	tanh
Hidden layer	two layer
Processing element of hidden one	6
Processing element of hidden two	6
Learning rate	0.4
Momentum rate	0.25
Epochs	8000

2.7 Determination of arsenic(III) in ground water samples from Ron Piboon District using ASV technique and data processing by artificial neural networks

Results of determination of arsenic(III) in ground water samples from Ron Piboon District, Nakhon Si Thammarat Province using ASV technique and the data of voltammograms used as input for predict a concentration of arsenic(III) by artificial neural networks were presented in Table 27.

Table 27 Determination of arsenic(III) in ground water samples from Ron Piboon District using ASV technique and data processing by ANN

Sample labeled No.	Concentration of arsenic(III) (ppb)
1	26.69(±0.71)
2	1.05(±0.12)
3	16.68(±1.09)
4	1.10(±0.04)
5	1.64(±0.07)

Results Obtained from Hydride Generation Atomic Absorption Spectrometric Technique

The sensitivity of this method shown as the slope of the calibration line was 0.0209 and the correlation coefficient of the corresponding dependence was 0.9946 in the range of 0 to 15 ppb arsenic(III). The calibration curve was shown in Figure 16. Results of determination of arsenic(III) in ground water samples from Ron Piboon District, Nakhon Si Thammarat Province using HG-AAS technique were presented in Table 28.

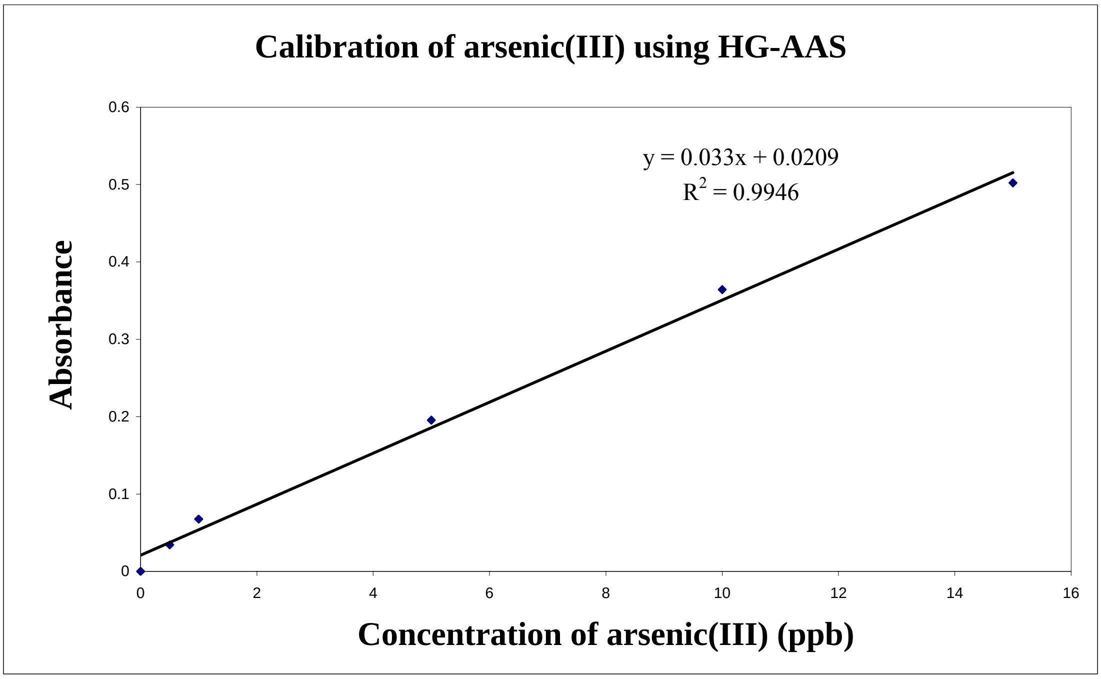

Figure 16 Calibration curve for arsenic(III) obtained from HG-AAS technique

Table 28 Determination of arsenic(III) in ground water samples from Ron Piboon District using HG-AAS technique.

Sample labeled No.	Arsenic(III) concentration found (ppb)
1	26.77(±0.03)
2	0.90(±0.02)
3	16.49(±0.01)
4	1.08(±0.02)
5	1.58(±0.01)

Comparison of Results Obtained from two Techniques

Table 29 Comparison of results obtained from ASV data processing by ANN and HG-AAS technique of determination of arsenic(III) in ground water samples from Ron Piboon District.

Sample labeled No.	Arsenic(III) concentration found (ppb)	
	ASV data processing by ANN	HG-AAS
1	26.69(±0.71)	26.77(±0.03)
2	1.05(±0.12)	0.90(±0.02)
3	16.68(±1.09)	16.49(±0.01)
4	1.10(±0.04)	1.08(±0.02)
5	1.64(±0.07)	1.58(±0.01)

Table 30 Significance test between two methods for determination of arsenic(III)

Sample labeled No.	t value		Results
	t cal	t table (p=0.05)	
1	0.24	2.18	accepted
2	2.32	2.36	accepted
3	0.44	2.57	accepted
4	0.92	2.36	accepted
5	1.52	2.36	accepted

CONCLUSIONS

The advantage of developed anodic stripping voltammetry (ASV) method using gold electrode as a working electrode was the use of mild conditions and very sensitive. Arsenic(III) was determined at a deposition potential of -0.2 V versus Ag/AgCl, deposition time of 180 s, scan rate of 80 mVs^{-1} and modulation amplitude of 0.1 V. A 5 M H_2SO_4 and 0.5 M HCl solution was used as the supporting electrolyte. An artificial neural network (ANN) was utilized for the analysis of the voltammogram data. The linear calibration curve of arsenic(III) was obtained in concentration range between 0.25 and 50 ppb with correlation coefficient of 0.9998. Relative standard deviation at 5 ppb was 3% (n=10) and the limit of detection was 0.25. This work shows that ASV is a viable low cost instrumental technique for the measurement and speciation of arsenic in groundwater at μgL^{-1} concentration.

This proposed method was applied to determine arsenic(III) in ground water samples collected from Ron Piboon District, Nakhon Si Thammarat Province. The results showed that two samples contain arsenic(III) concentration more than maximum contaminant level (MCL) of EPA and WHO recommendation. The results obtained from integrated ASV and ANN agree very well with those obtained from hydride generation-atomic absorption spectrometry (EPA Method 1632).

SUGGESTION FOR FURTHER WORKS

In this work shows that ASV can be used for the determination of copper(II) with the same conditions as the determination of arsenic(III). For this reason, further studies are to develop method for simultaneous determination of arsenic(III) and copper(II) by differential pulse anodic stripping voltammetry(DPASV) and to use the artificial neural network to predict both the arsenic(III) and copper(II) contents in water samples.

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APPENDIX

APPENDIX A

Introduction to neural networks

1. What is a neural network?

An Artificial Neural Network (ANN) is an information processing paradigm that is inspired by the way biological nervous systems, such as the brain, process information. The key element of this paradigm is the novel structure of the information processing system. It is composed of a large number of highly interconnected processing elements (neurons) working in unison to solve specific problems. ANNs, like people, learn by example. An ANN is configured for a specific application, such as pattern recognition or data classification, through a learning process. Learning in biological systems involves adjustments to the synaptic connections that exist between the neurones. This is true of ANNs as well.

2. Historical background

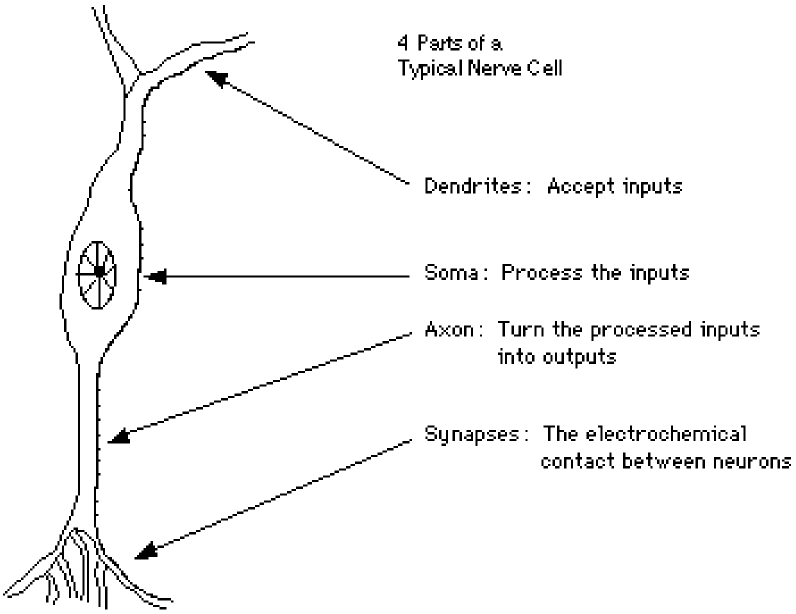
Neural network simulations appear to be a recent development. However, this field was established before the advent of computers, and has survived at least one major setback and several eras.

Many important advances have been boosted by the use of inexpensive computer emulations. Following an initial period of enthusiasm, the field survived a period of frustration and disrepute. During this period when funding and professional support was minimal, important advances were made by relatively few researchers. These pioneers were able to develop convincing technology which surpassed the limitations identified by Minsky and Papert. Minsky and Papert, published a book (in 1969) in which they summed up a general feeling of frustration (against neural networks) among researchers, and was thus accepted by most without further analysis. Currently, the neural network field enjoys a resurgence of interest and a corresponding increase in funding.

The first artificial neuron was produced in 1943 by the neurophysiologist Warren McCulloch and the logician Walter Pits. But the technology available at that time did not allow them to do too much.

3. Artificial neurons and how they work

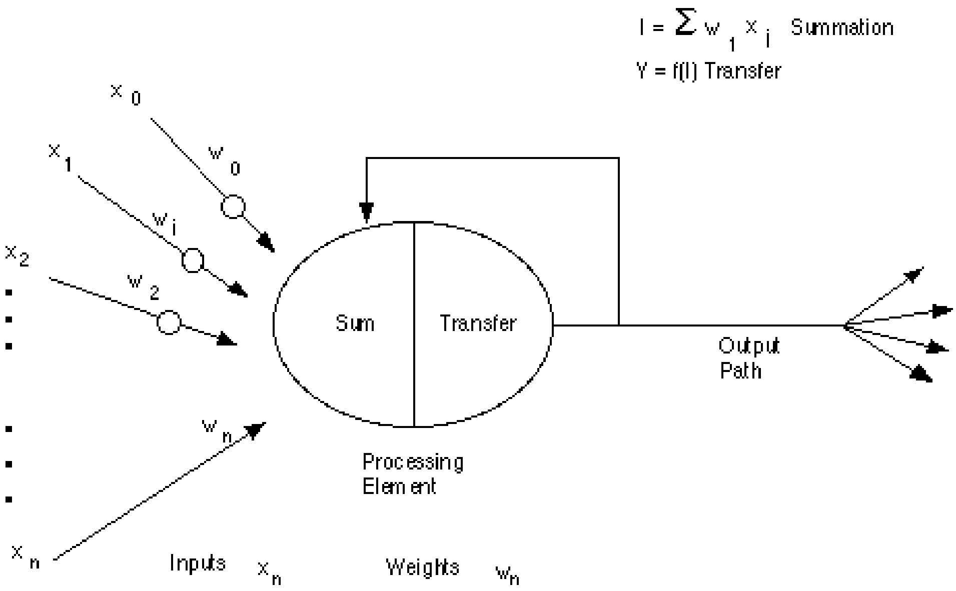
The fundamental processing element of a neural network is a neuron. This building block of human awareness encompasses a few general capabilities. Basically, a biological neuron receives inputs from other sources, combines them in some way, performs a generally nonlinear operation on the result, and then outputs the final result. Appendix Figure A1 shows the relationship of these four parts.



Appendix Figure A1 A simple neuron

Within humans there are many variations on this basic type of neuron, further complicating man's attempts at electrically replicating the process of thinking. Yet, all natural neurons have the same four basic components. These components are known by their biological names - dendrites, soma, axon, and synapses. Dendrites are hair-like extensions of the soma which act like input channels. These input channels receive their input through the synapses of other neurons. The soma then processes these incoming signals over time. The soma then turns that processed value into an output which is sent out to other neurons through the axon and the synapses.

The basic unit of neural networks, the artificial neurons, simulates the four basic functions of natural neurons. Appendix Figure A2 shows a fundamental representation of an artificial neuron.

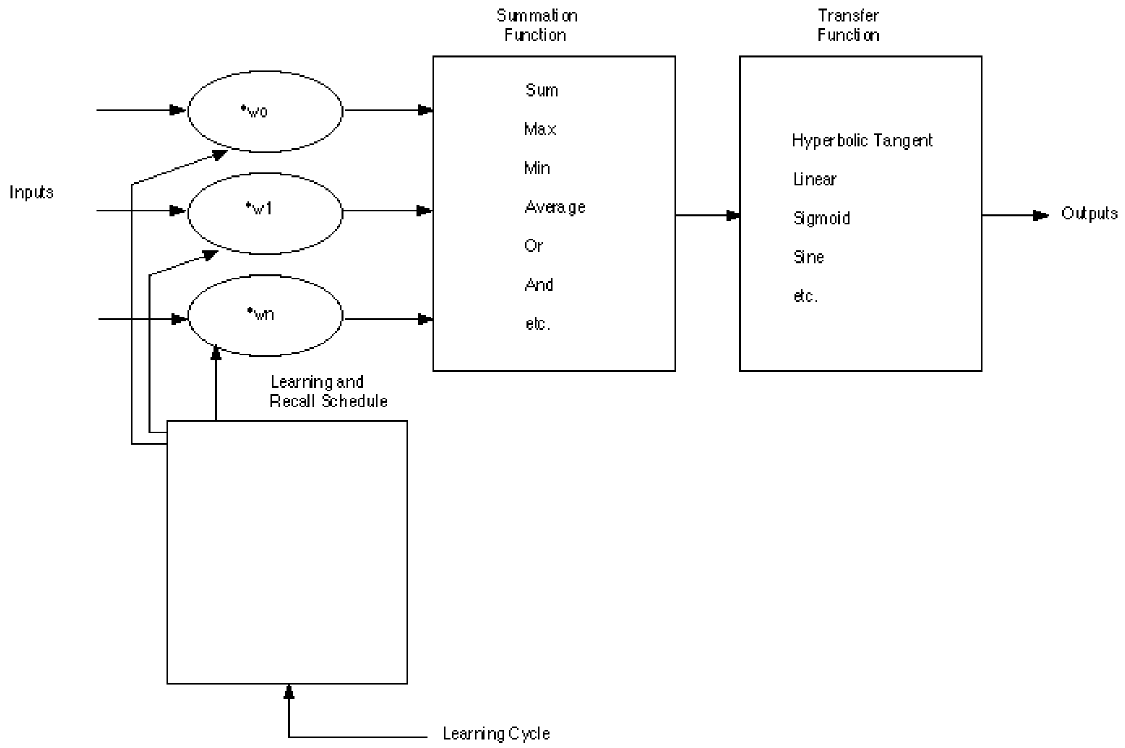


Appendix Figure A2 A basic artificial neuron

In Appendix Figure A2, various inputs to the network are represented by the mathematical symbol, x_n. Each of these inputs are multiplied by a connection weight. These weights are represented by w_n. In the simplest case, these products are simply summed, fed through a transfer function to generate a result, and then output. This process lends itself to physical implementation on a large scale in a small package. This electronic implementation is still possible with other network structures which utilize different summing functions as well as different transfer functions.

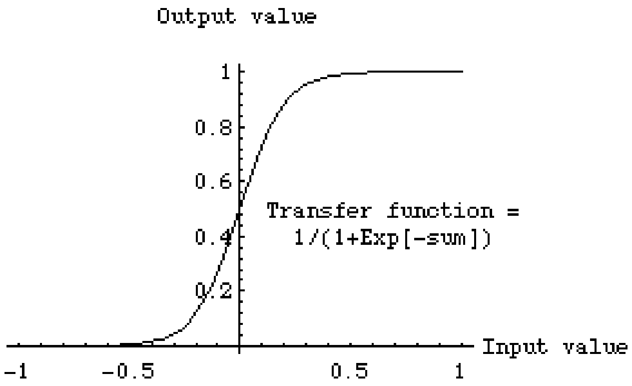
4. Electronic implementation of artificial neurons

In currently available software packages these artificial neurons are called "processing elements" and have many more capabilities than the simple artificial neuron described above. Appendix Figure A3 is a more detailed schematic of this still simplistic artificial neuron.



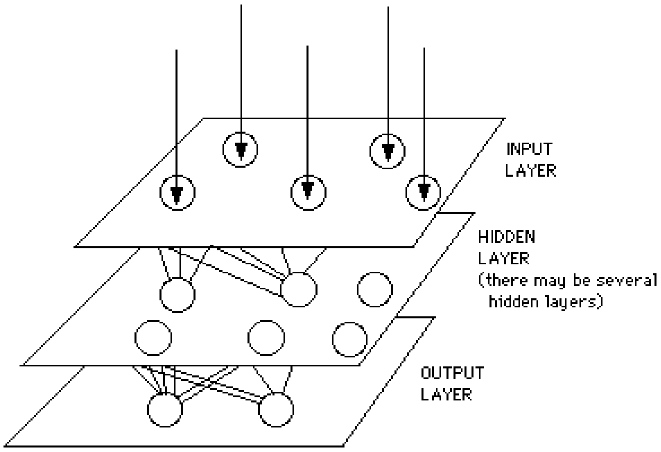
Appendix Figure A3 A model of a "Processing Element"

In Appendix Figure 3, inputs enter into the processing element from the upper left. The first step is for each of these inputs to be multiplied by their respective weighting factor (w_n). Then these modified inputs are fed into the summing function, which usually just sums these products. Yet, many different types of operations can be selected. These operations could produce a number of different values which are then propagated forward. The output of the summing function is then sent into a transfer function. This function then turns this number into a real output via some algorithm. It is this algorithm that takes the input and turns it into a zero or a one, a minus one or a one, or some other number. The transfer functions that are commonly supported are sigmoid, sine, hyperbolic tangent, etc. This transfer function also can scale the output or control its value via thresholds. The result of the transfer function is usually the direct output of the processing element. An example of how a transfer function works is shown in Appendix Figure A4. This sigmoid transfer function takes the value from the summation function, called sum in the Appendix Figure A4, and turns it into a value between zero and one.



Appendix Figure A4 Sigmoid transfer function

Finally, the processing element is ready to output the result of its transfer function. This output is then input into other processing elements, or to an outside connection, as dictated by the structure of the network. Basically, all artificial neural networks have a similar structure or topology as shown in Appendix Figure A5



Appendix Figure A5 A simple neural network diagram

5. Feedforward, Back-Propagation.

In order to train a neural network to perform some task, we must adjust the weights of each unit in such a way that the error between the desired output and the actual output is reduced. This process requires that the neural network compute the error derivative of the weights (EW). In other words, it must calculate how the error changes as each weight is increased or decreased slightly. The back propagation algorithm is the most widely used method for determining the EW.

The back-propagation algorithm is easiest to understand if all the units in the network are linear. The algorithm computes each EW by first computing the EA, the rate at which the error changes as the activity level of a unit is changed. For output units, the EA is simply the difference between the actual and the desired output. To compute the EA for a hidden unit in the layer just before the output layer, we first identify all the weights between that hidden unit and the output units to which it is connected. We then multiply those weights by the EAs of those output units and add the products. This sum equals the EA for the chosen hidden unit. After calculating all the EAs in the hidden layer just before the output layer, we can compute in like fashion the EAs for other layers, moving from layer to layer in a direction opposite to the way activities propagate through the network. This is what gives back propagation its name. Once the EA has been computed for a unit, it is straight forward to compute the EW for each incoming connection of the unit. The EW is the product of the EA and the activity through the incoming connection.

APPENDIX B

Chemical list

Appendix Table B1 List of chemicals used in this study

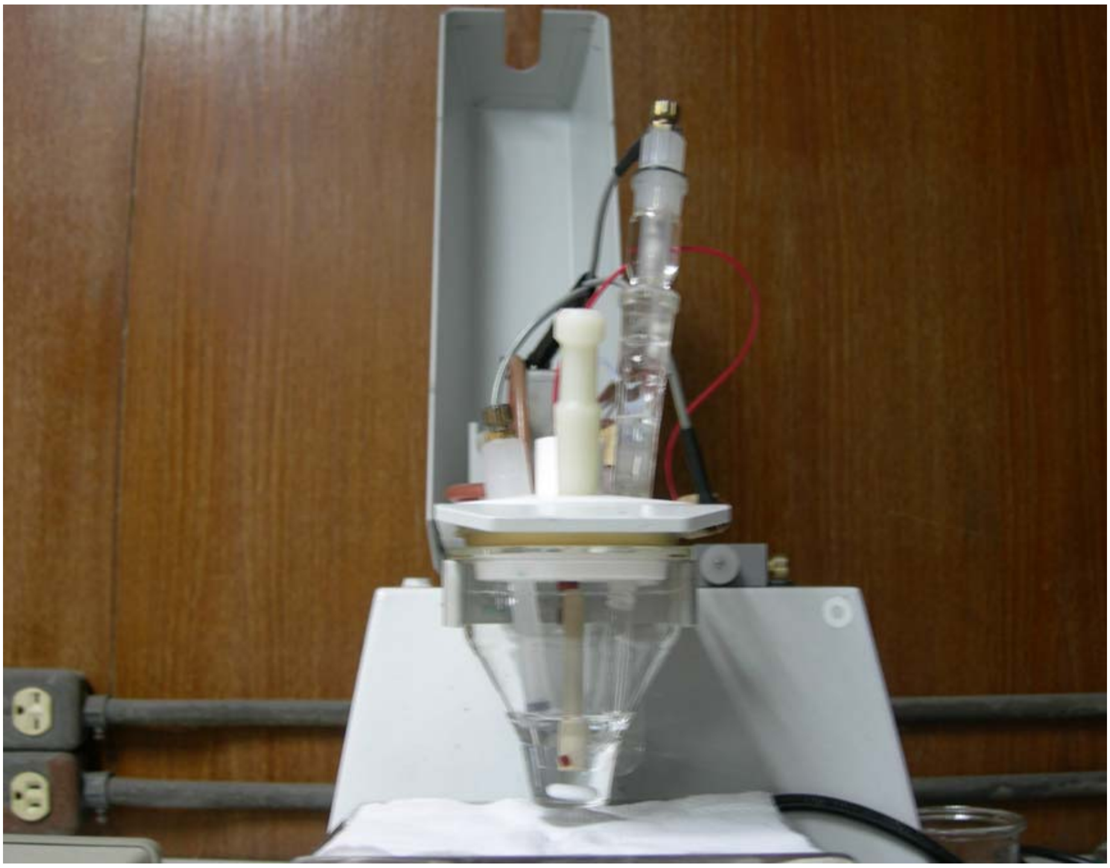
Chemicals	Formula	Molecular weight	Company	Grade
di-Arsenic trioxide	As_2O_3	197.84	Merck (Darmstadt, Germany)	Pro analysis
Ammonium-pyrrolidindithiocarbamate	$C_5H_5NS_2NH_4$	164.29	Fluka (Buchs SG, Switzerland)	Purum p.a.
Cadmium sulfate	$3CdSO_4.8H_2O$	769.51	Merck (Darmstadt, Germany)	Pro analysis
Copper sulfate	$CuSO_4.5H_2O$	249.68	Mallinckrodt (ST.Louis,Missouri,USA)	Analytical reagent
Cupferron	$C_6H_5N(NO)ONH_4$	155.16	May&Baker (Dagenham, England)	Laboratory chemicals
Hydrazinium sulfate	$NH_2NH_2.H_2SO_4$	130.12	Fluka (Buchs SG, Switzerland)	Puriss p.a.
Hydrochloric acid	HCl	36.50 (d=1.19 g/cm^3)	Merck (Darmstadt, Germany)	Pro analysis

Appendix Table B1 (Cont'd)

Chemicals	Formula	Molecular weight	Company	Grade
Iron(II)sulfate	$FeSO_4.7H_2O$	278.02	Unilab (Australia)	Laboratory reagent
Lead(II)nitrate	$Pb(NO_3)_2$	331.21	Merck (Darmstadt, Germany)	Pro analysis
Nitric acid	HNO_3	63.01 (d=1.42 g/cm^3)	Lab scan (Ireland)	Analytical reagent
Sodium hydroxide	NaOH	40.00	Merck (Darmstadt, Germany)	Pro analysis
Sulfuric acid	H_2SO_4	98.08 (d=1.84 g/cm^3)	Merck (Darmstadt, Germany)	Extra pure

APPENDIX C

Appendix Figure C1 Potentiostat (PGSTAT20, Metrohm)



Appendix Figures C2 663 VA stand (Metrohm) and electrochemical cell

Appendix Figures C3 Electrochemical cell and electrodes from Metrohm

Appendix Figure C4 Hydride generation-atomic absorption spectrometer (Spectra AA880Z,Varian)

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