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# OPTIMIZATION AND VALIDATION OF A DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRIC (DPASV) METHOD FOR TRACE ANALYSIS OF INORGANIC ARSENIC IN CONTAMINATED WATER

**Maritess L. Magalona[1], Milagros M. Peralta[1],**
**Marivic S. Lacsamana[1], Veronica C. Sabularse[1] and Constancio C. de Guzman[2]**
[1]Institute of Chemistry, College of Arts and Sciences,
[2]Institute of Crop Science, College of Agriculture and Food Science,
University of the Philippines Los Baños, College Laguna, 4031
Corresponding author: mmperalta@up.edu.ph

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## ABSTRACT

The maximum contamination limit set by the U.S. Environmental Protection Agency (EPA) for arsenic in drinking water is 10 ppb in view of the adverse effects of chronic arsenic exposure on human health. Hence, there is a need to develop an inexpensive, field-operable method that can quantify arsenic at or below this concentration to ensure compliance with EPA regulations without exposure of the analyst to toxic arsine gas. An electroanalytical method was optimized and validated to analyze trace inorganic arsenic as As(III) and total As (As(III) + As(V)) using differential pulse anodic stripping voltammetry with a gold disk as the working electrode, Pt/Ti rod as the auxiliary electrode, and Ag/AgCl as the reference electrode. The study was conducted at the Institute of Chemistry, University of the Philippines Los Baños from June 2015 to October 2016. The electroanalytical method was found to be precise and sensitive based on the resulting RSD values (< 13%). It also hada satisfactory percent recovery of 91% for As(III) and 81% for As(V). The limit of detection of As(III) and As(V) were 2.24 and 6.96 ppb, respectively while the limit of quantification of As(III) and As(V) were 7.49 and 23.19 ppb, respectively. The total arsenic content of groundwater samples obtained by this method was validated with inductively coupled plasma – optical emission spectrophotometry, and statistical analysis using the t-test showed that the two methods were not significantly different. This inexpensive and rapid method allows for speciation of the arsenic species found in field water samples and will be a great boon for monitoring water quality for farming communities that rely on raw groundwater for cooking and drinking.

**Key words:** electrochemical methods, arsenic speciation analysis

## INTRODUCTION

Arsenic is a metalloid which is ubiquitous in nature (Mandal and Suzuki 2002). It ranks 20$^{th}$ in abundance in the earth's crust, 14$^{th}$ in seawater, and 12$^{th}$ in the human body (Mandal and Suzuki 2002, Khan and Lo 2011). Because of its wide abundance, humans are always exposed to it in food, water, air, and soil. In the past century, the use of chemical fertilizers as part of the Green Revolution included the widespread use of rock phosphate in fertilizers as well as the use of herbicides and pesticides containing phosphorus. This has also resulted in an increased exposure to arsenic in agricultural areas because arsenic is a natural contaminant of phosphate minerals and rock phosphate. In addition, phosphate affects mobility of arsenic in the soil and its mobilization into the water supply

(Signes-Pastor et al. 2007, Curtis and Smith 2002, Macedo et al. 2009, Jayasumana et al. 2015). Common forms of arsenic include organic and inorganic species with (+5) or (+3) oxidation states as well as arsine gas ($As^{-3}$). The inorganic forms (As(III) and As(V)) are more toxic than the organic ones, and As(III) is much more toxic than As(V). The most toxic form of arsenic is arsine gas (National Center for Biotechnology Information. PubChem Compound 23969; accessed Mar. 4, 2018). Thus, it is not enough to determine the total arsenic concentration of the water samples. Speciation analysis must be conducted as well in order to get a clearer picture of the extent of the arsenic contamination problem. Although colorimetric kits using test strips are available that claim the ability to detect arsenic levels at below the maximum contamination limit, the methods rely on the conversion of inorganic arsenic ions to highly toxic arsine gas and its detection by color development in the presence of a test strip soaked in mercuric bromide (Gary 2001).

Arsenic is in the World Health Organization's (WHO) list of ten chemicals of greatest public health concern (WHO 2010). The arsenic content of water used for drinking and cooking is highly regulated because of the high incidence of chronic poisoning due to arsenic contamination of drinking water (Mandal and Suzuki 2002, Khan and Ho 2011). The current recommended exposure level of arsenic in drinking water is set by the WHO and the U.S. Environmental Protection Agency (EPA) at 10 µg/L or 10 ppb (Muñoz and Palmero 2005). Above this level, symptoms manifest as a sign of acute toxicity, such as gastrointestinal discomfort, vomiting, diarrhea, bloody urine, anuria, shock, convulsions, coma, and death. On the other hand, results of chronic toxicity vary from non-malignant skin lesions like hyperpigmentation, hyperkeratosis, and hypopigmentation (Hughes et al. 2011) to cancer of skins and various internal organs (Mazumder 2000).

Several areas around the world have been found to have arsenic levels that pose significant toxicological concerns. One of the largest outbreaks of arsenic poisoning occurred in Bangladesh and was traced to the use of groundwater sources for drinking, cooking and irrigation (Mandal and Suzuki 2002, Khan and Ho 2011). Other countries that have known to have high levels of arsenic in water include India, Cambodia, the Lao People's Democratic Republic, Myanmar, Pakistan, Nepal, Cambodia, Vietnam, China, Iran, Thailand, Taiwan, England, Chile, Mexico, Argentina, Canada, and United States, (Mandal and Suzuki 2002, Feeney and Kounaves 2002, Khan and Ho 2011). Recently, an increasing number of cases of arsenicos is has been recorded by the Department of Health (DOH) in the Philippines. As of June 2016, 123 arsenicos is cases were recorded in Central Luzon (Santos 2016, Sun.Star Manila 2016). Farming communities are especially vulnerable because these areas rely mainly on raw ground water for cooking, drinking and irrigation.

The arsenic release potential is highly correlated with three soil characteristics or hydrogeological situations (Alaerts and Khouri 2004): (a) peaty or peaty-clayey soils with high humic organic content and a high water table containing arsenopyrite crystals; (b) young volcanic deposits or thermal water sources where elevated dissolved arsenic concentrations can well exceed 1 ppm; and (c) loamy and clayey deposits (especially in deltaic areas) that may contain arsenic in dissolved state and/or adsorbed onto clay particles.

It is necessary to drastically reduce heavy metal contamination in water used for food, agricultural and household purposes to prevent human health risks and environmental damage. Crops grown in contaminated soils may bioaccumulate pollutants present in the soil and from the water source itself (Abedin 2002, Dahal 2008). Since groundwater contributes largely to the irrigation needs of the several countries, widespread use of arsenic contaminated irrigation water may possibly lead to issues of food security and degradation of soils (Naik 2015). Until very recently, arsenic in the form of roxarsone and its derivatives (e.g. nitarsone, carbarsone or arsanilic acid) were given as feed additives in the United States for chickens, turkeys, and pigs to increase disease resistance, increase feed efficiency, and promote growth (Larsen 2015). Even, poultry growers in the Philippines include some of these supplements as feed additives for their chickens. Pfizer Inc., the makers of roxarsone

(trade name: 3-Nitro*) issued a voluntary withdrawal of roxarsone in the market in the light of studies done by the US FDA that showed that the levels of inorganic arsenic were significantly higher in the livers of chickens that were fed this supplement (DOH/FDA & DA-BAI Advisory No. 2011-01 2011).

With the reduction of the maximum contamination limit (MCL) for arsenic from 50 ppb to 10 ppb in drinking water, the development of simple, cost-effective methods for trace detection of this toxic metalloid has become urgent. Methods that involve the production of arsine gas, the most toxic form of arsenic, are not desirable. These methods include the colorimetric test kits (Gary 2001) and differential pulse cathodic stripping voltammetry (DPCSV) (Hung 2004). This study focuses on the development of differential pulse anodic stripping voltammetric (DPASV) method for trace arsenic analysis as As(III) that can analyze arsenic levels below 10 ppb. Voltammetry is the widely used analytical technique for arsenic due to its low maintenance cost, high selectivity and sensitivity (Hung et al. 2004), capability for speciation of inorganic arsenic (Song and Swain 2007), and suitability for screening water samples since it can be taken into the field for field analysis (Salaun 2007). Although arsenic can be detected by several methods, which include atomic fluorescence spectrometry coupled with hydride generation system, inductively coupled plasma – optical emission spectrophotometry (ICP – OES), and inductively coupled plasma – mass spectrometry (ICP – MS),these methods however, require high maintenance costs and a high level of expertise for the analyst. On the other hand, the U.S. EPA also accredits the use of anodic stripping voltammetry for arsenic analysis in aqueous solutions (U.S. EPA Method 7063 1996), the method which was developed in this study using an inexpensive potentiostat that can be field operable. In addition, various reducing agents were explored to reduce arsenate ions to arsenite and enable subsequent arsenic speciation analysis.

## MATERIALS AND METHODS

### Chemicals

The reagents used were ensured to be of analytical grade. Sodium hydroxide pellets and concentrated sulfuric acid were from RCL LabScan. L-cysteine was from Sigma Aldrich. Ferric sulfate hydrate, 2-propanol and n-hexane were from Scharlau. Analytical grade hydrochloric acid and ammonium hydroxide were from JT Baker while glacial acetic acid was from Macron Fine Chemicals. For the preparation of the aqueous solutions, deionized distilled water (conductivity = $5\times10^{-6}$ S/m) was used. As(III) and As(V) solutions were prepared from sodium (meta)arsenite (Sigma Aldrich) and sodium arsenate heptahydrate (JT Baker), respectively. Standard As(III) and As(V) solutions as well as other chemical reagents (like 1 M HCl) used were prepared using deionized distilled water(conductivity = $5\times10^{-6}$ S/m). The prepared standard solutions of As (III) and As(V) were used to optimize and validate the DPASV method, and to determine the exact concentration of As(III) and As(V) in the groundwater samples. Fifty ppb of As(III) and As(V) were used to optimize and validate the DPASV method while 0 to 100 ppb of As(III) and As(V) were used to determine the exact concentration of As(III) and As(V) in the groundwater samples.

### Equipment

Voltammetric analyses were done using eDAQ potentiostat and e-corder 410 (eDAQ Southeast Asia, Thailand) with ET076 gold disk (eDAQ Southeast Asia, Thailand) as the working electrode, ET078 Pt/Ti rod (eDAQ Southeast Asia, Thailand) as the auxiliary electrode, and BASi Ag/AgCl (BASi, Indiana, U.S.A.) as the reference electrode. Each analysis was analyzed in triplicate.

### Conditioning of gold electrode

Prior to Differential Pulse Anodic Stripping Voltammetry (DPASV) analysis, the gold electrode was conditioned using the protocol of Salaun et al. (2007) by electrochemically cycling at least ten times between 0 and 1.5 V at a scan rate of 0.1 V/s in 0.5 M $H_2SO_4$.

**Cyclic voltammetry (CV) of As(III)**

To determine the position of the oxidation and reduction peaks of As (III), cyclic voltammetry of 1 ppm As(III) solution was determined using 1M HCl as the supporting electrolyte and the potential was scanned at a range of -0.5 to +0.50 V with a scan rate of 0.1 V/s.

**Optimization of DPASV parameters**

Initial DPASV settings used for optimization were the following: deposition potential = -0.25 V, deposition time = 180 s, scan rate = 0.05 V/s, and rest time = 10 s. Parameters that were optimized included HCl concentration (0.1, 0.5, 1, and 2 M), deposition potential (-0.10 V to -0.35 V in 0.05 V increments), deposition time (60, 120, 180, 240, and 300 s), equilibration time (5, 10, and 15 s), scan rate (0.025, 0.05 and 0.07 V/s), and chemical reducing agent (sodium sulfite, sodium meta-bisulfite, sodium thiosulfate, and L-cysteine) for As(V) to As(III). The working solution for each optimization was 50 ppb As(III).

**Validation of DPASV method**

*Linearity and Precision.* The linearity of the response of As(III) and As(V) was determined from 0 to 100 ppb arsenic using standard solutions and evaluated using a constructed calibration curve to calculate the correlation coefficient ($r^2$), slope (m), and intercept (b) values. The sensitivity and precision of the instrument response were evaluated by replicating the calibration curve five times at different days. Precision was calculated as the relative standard deviation (RSD).

$$\text{RSD (\%)} = \frac{\text{standard deviation}}{\text{mean}} \times 100 \qquad \text{(Equation 1)}$$

*LOD and LOQ.* Based on the standard deviation (s) of ten readings of the blank, LOD, and LOQ were calculated as follows:

$$\text{LOD} = \frac{3s}{\text{m}}; \quad \text{LOQ} = \frac{10s}{\text{m}} \qquad \text{( (Equation 2)}$$

where: s = standard deviation of the blank
m = slope of the calibration curve

*Accuracy by percent recovery*

The accuracy of the method was evaluated using percent recovery. This was done using a spiked sample prepared by adding 50 ppb As(III) or reduced As(V) to the groundwater sample. Three replicates were done. The formula for percent recovery is shown below:

$$\text{\% recovery} = \frac{[\text{spiked sample}]-[\text{original sample}]}{50\ \text{ppb}} \times 100 \qquad \text{(Equation 3)}$$

This method of single point addition was also performed for arsenic analysis of water samples that had very low concentrations.

**Water sampling**

Prior to obtaining groundwater samples, purging of the pump source was carried out according to standard protocols (USGS Field Manual 2006). The water pump was pumped continuously for ten minutes so that interfering substances in the analysis were removed. The clean bottled container was filled with the water sample to the brimand was put in an ice box. Acidification of the water sample (1 mL concentrated HCl (JT Baker, analytical grade) per 500 mL water sample) was done in the laboratory in order to preserve the sample. Prior to DPASV and ICP – OES analysis, water samples were digested at 85 °C for 1 hour using High Performance Microwave Digestion System – Microwave Reactor 33 (Milestone Ethos UP)to ensure the removal of organic matter and suspended solids (US EPA Method3015A 2015).

**Arsenic analysis of groundwater samples using DPASV and validation using ICP – OES**

Groundwater samples from Laguna were acidified with HCl to a final concentration of 1 M. The As(III) and total As contents were determined using the optimized parameters of DPASV. The samples were analysed in triplicate.

The total arsenic content obtained using DPASV method was validated using ICP – OES method following US EPA Method 200.7 (Martin et al. 1994). The samples were analyzed in triplicate.

## RESULTS AND DISCUSSION

Voltammetric determination of arsenic generally makes use of a gold working electrode, either as solid gold electrode (Forsberg 1975, Hung et al. 2004) or gold film electrode (Davis et al. 1978, Sun et al. 1997) because it is the most suitable electrode for the analysis. Mercury electrodes using cathodic stripping analysis of arsenic give relatively poor sensitivity and possibly large error in analysis due to the presence of interferences from some ions (Muñoz and Palmero 2005). Anodic stripping analysis of arsenic, with gold and platinum as working electrodes, gives high sensitivity. However, a limitation of this analysis involves the evolution of hydrogen gas at a more negative deposition potential that may hinder the deposition of arsenic on the surface of the working electrode (Giacomino et al. 2011). Gold was found to be superior to platinum as the working electrode due to its higher hydrogen overvoltage compared to platinum (Forsberg 1975, Hung et al. 2004, Muñoz and Palmero 2005).

Macroelectrodes or normal size electrodes with few millimetres in diameter are still the most popular commercially available working electrodes because these are easy to handle (Mays 2013) and maximize the amount of analyte reduced while minimizing the deposition time (Copeland and Skogerboe 1974). However, several studies encountered problem with regards to the reproducibility of the arsenic signals (peak heights) due to saturation and formation of oxide films on the surface of the gold electrode (Muñoz and Palmero 2005, Giacomino et al. 2011). To avoid this problem, special treatment procedures like polishing using commercially available polishing powder materials were used to regenerate the surface of the electrode.

In this study, reproducible peak heights of As(III) were obtained by conditioning the electrode prior to analysis. This conditioning step involved at least 10 repetitions of electrochemical cycling between 0 and 1.5 V at a scan rate of 0.1 V/s in 0.5 M $H_2SO_4$at the start of the analysis and by repeating the electrochemical cycling five times in between sample analysis. In addition, similar to the studies of Salaün et al. (2007) and Alves et al. (2011), well-defined As(III) peaks were obtained in this study even without deoxygenating the sample solution with $N_2$ gas. Figure 1 shows the voltammograms of 5 replicates of 50 ppb As(III) solution.

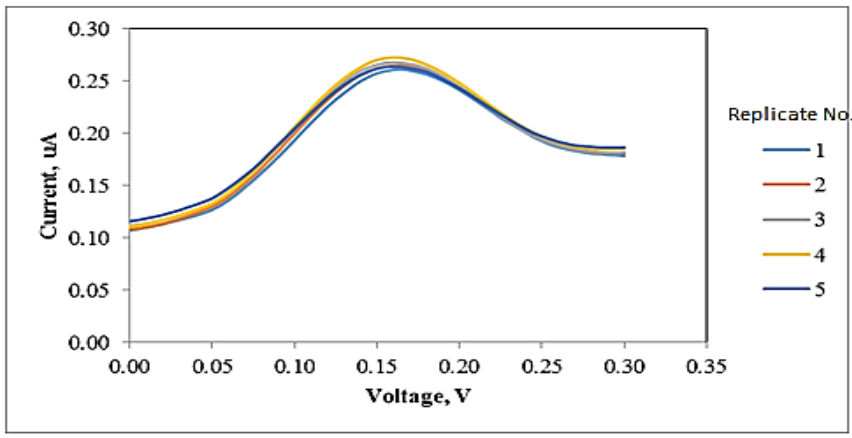



**Fig. 1.** Voltammogram of five replicates of 50 ppb As(III) in 1 M HCl by DPASV using the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode, and Pt/Ti auxiliary electrode. DPASV parameters: deposition potential: -0.25 V; deposition time: 180 s; rest time: 10 s; scan rate: 0.050 V/s.

**Optimization of parameters for the DPASV analysis of As(III)**

Prior to the optimization experiments, cyclic voltammetry was conducted in order to determine the expected position of the peak in the DPASV analysis. Figure 2 shows the cyclic voltammogram for As(III). The reduction and oxidation peak currents occurred at around -0.28 V and 0.18 V, respectively. The value of reduction peak tells us that the most efficient deposition of As(III) towards the surface of the electrode occurs at around -0.28 V. The oxidation peak value is in agreement with the reported values of oxidation peak of As(III) (U.S. EPA 1996, Švancara et al. 2002, Song and Swain 2007).

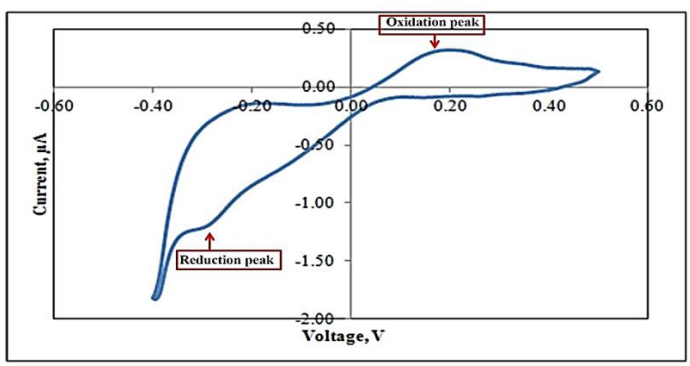



**Fig. 2.** Cyclic voltammogram of 1 ppm As(III) in 1M HCl obtained using the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode, and Pt/Ti auxiliary electrode. and the following default parameters: scan rate: 0.1 V/s; initial potential: 0.5 V; final potential: -0.4 V.

In order to adapt the DPASV method to ppb concentrations of As(III), five parameters were optimized: HCl concentration, deposition potential, deposition time, rest time, scan rate, and reducing agent for the conversion of As(V) to As(III). The optimization experiments were done using 50 ppb As(III) in HCl solution because 50 ppb arsenic was the maximum contamination limit previously set by the WHO and US EPA. Several studies established that the best electrolyte solution for arsenic

analysis using gold electrode is HCl (Forsberg et al. 1975, Salaün et al. 2007) due to the formation of chloride complexes. Chlorides form strong complexes with As(III) as shown in Equation 4.

$$H_3AsO_3 + HCl \rightleftharpoons As(OH)_2Cl + H_2O$$
$$H_3AsO_3 + 2\,HCl \rightleftharpoons As(OH)Cl_2 + 2\,H_2O \qquad \text{(Equation 4)}$$
$$H_3AsO_3 + 3\,HCl \rightleftharpoons AsCl_3 + 3\,H_2O$$

The peak height increased with increasing concentrations of HCl. $As(OH)_2Cl$ and $As(OH)Cl_2$ are thought to be the arsenic species involved in the electron transfer reaction on the electrode(Fig. 3a). Therefore, the efficiency of deposition of As(III) increased with increasing concentration of HCl due to the increased formation of $As(OH)_2Cl$ and $As(OH)Cl_2$in solution (Sun et al. 1997, Muñoz and Palmero 2005). When 2 M HCl was used as electrolyte, a decrease in peak current response was noted. This decrease in peak current indicates that the electrode has been saturated with adsorbed chlorides (Muñoz and Palmero 2005). Thus, the optimized concentration of 1 M HCl, which is also the concentration of HCl used in the study of Song and Swain (2007), was used throughout the analysis.

The sensitivity of the DPASV method depends on the amount of analyte deposited on the surface of the electrode. Thus, the length (deposition time) and the efficiency of deposition (deposition potential) were optimized to obtain the highest possible sensitivity (Sun et al. 1997). The optimum deposition potential and deposition time were -0.25 V and 180 s (Fig. 3b and 3c).

Rest time was not often optimized in several studies. This study took into consideration the importance of rest time since optimum peak height can only be obtained when the solution reaches equilibrium after deposition. The optimum rest time for the analysis was 10 seconds (Fig. 3d).

Scan rate is the primary parameter for maximizing the stripping current (Copeland and Skogerboe 1974). In this study, differential pulse was applied during stripping. As the voltage pulse is applied, the Faradaic current is generated. Optimum scan rate must be obtained to get good resolution of the voltammogram. Faster scan rates produce rapid and continuous change in potential that results to non-Faradaic current. The non-Faradaic current produces high background that degrades the signal-to-noise ratio (Copeland and Skogerboe 1974). Lower scan rates produce good peak resolution but smaller peak heights (Forsberg et al. 1975). As shown in Figure 3e, the optimum scan rate for the analysis was determined to be 0.05 V/s, which was the same as the scan rate used by Rasul et al. (2002).

The total arsenic in an aqueous sample is the summation of As(III) and As(V).To analyze As(V), it must be electrochemically reduced at a highly negative potential, which is at -1.2 V (Salaun et al. 2007) or chemically reduced to As(III) (Song and Swain 2007). The gold electrode used in the arsenic analysis cannot withstand highly negative potentials because of the tendency of hydrogen overvoltage (Forsberg et al. 1975), thus electrochemical reduction was not an option. Several chemical reducing agents that have been used to reduce As(V) to As(III).include sodium thiosulfate (Junsomboon et al. 2009), sodium sulfite (Song and Swain 2007), sodium meta-bisulfite (He et al. 2004), and L-cysteine (Svancara et al. 2002).

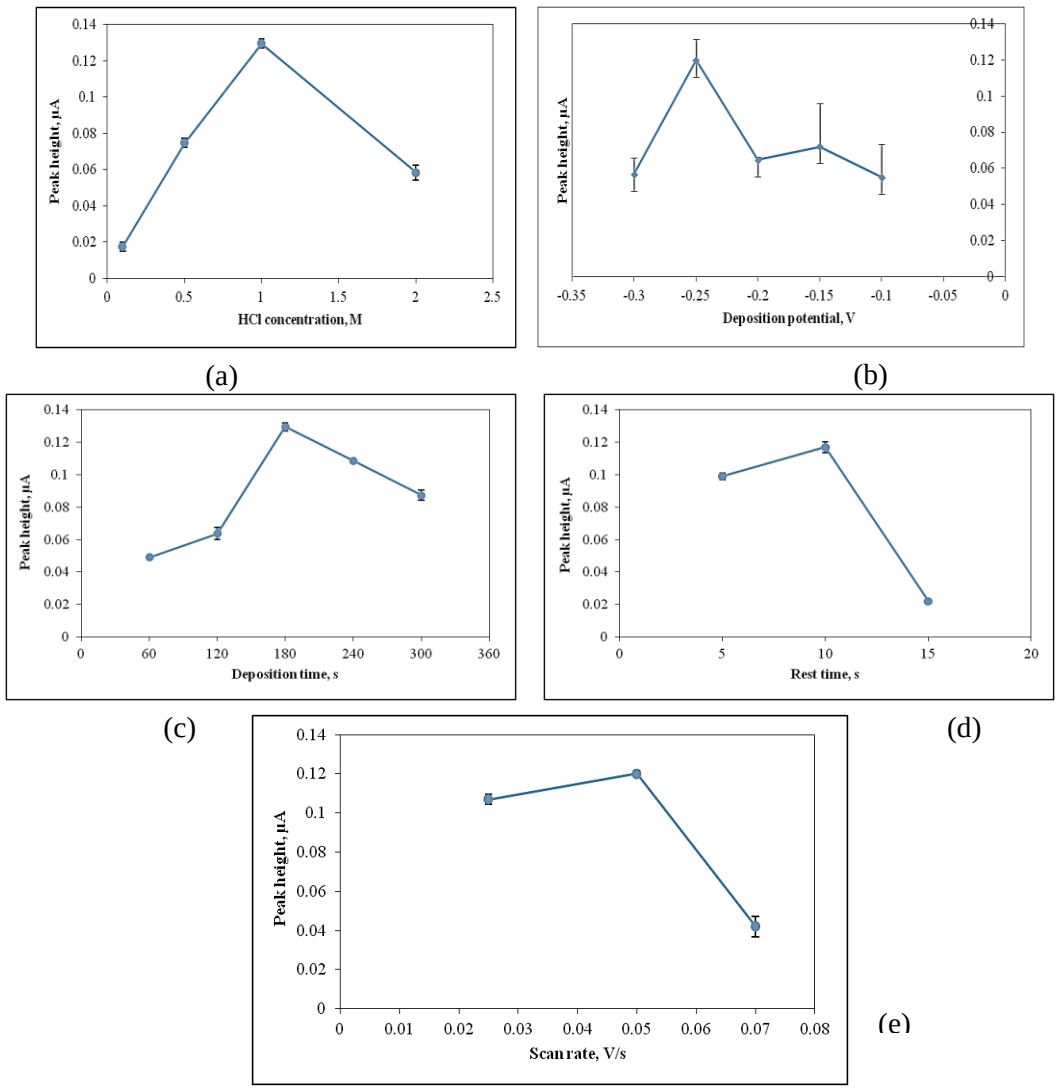
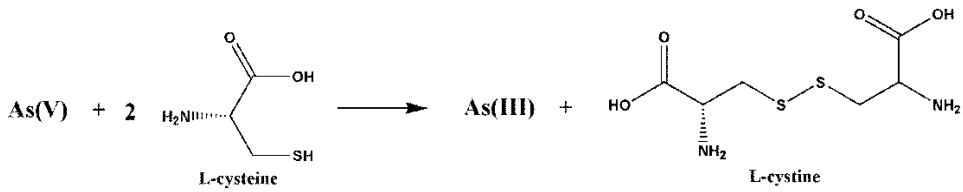



**Fig. 3.** Optimization of DPASV parameters: (a) HCl concentration, (b) deposition potential, (c) deposition time, (d) rest time, and (e) scan rate. Default DPASV parameters: deposition potential: -0.25 V; deposition time: 180 s; rest time: 10 s; scan rate: 0.050 V/s. Results were obtained using the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode, and Pt/Ti auxiliary electrode. Results are shown with error bars; 3 replicates; 3 readings per replicate.

The standard solution of 50 ppb As(V) did not show a peak in its voltammogram as expected (Fig. 4). All the above-mentioned reducing agents were tested in this study but problems were encountered, resulting in inconsistent DPASV results and poor peak response for most of the runs. Among these reducing agents, only L-cysteine from the method of Švancara et al. (2002) consistently gave good As(III) signal in DPASV. L-cysteine oxidizes to L-cystine while reducing As(V) to As(III) as shown in Equation 5 (Švancara et al. 2002).




(Equation 5)

The voltammogram of 50 ppb As(V) that was reduced by L-cysteine is also shown in Figure 4. The oxidation peak shifted to the positive direction relative to As(III) solution without L-cysteine (Fig. 2) and had a value of 0.28 V, consistent with the reported peak value of reduced As(V) of Švancara et al. (2002).

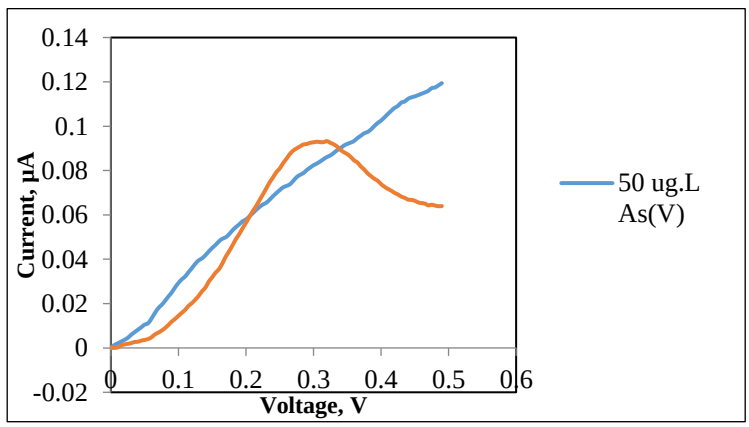



**Fig. 4.** Voltammogram of 50 ppb As(V), treated and not treated with L-cysteine. Results were obtained using the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode, and Pt/Ti auxiliary electrode. DPASV parameters: electrolyte: 1 M HCl; deposition potential: -0.25 V; deposition time: 180 s; rest time: 10 s; scan rate: 0.05 V/s.

The summary of the optimized parameters for arsenic analysis is shown in Table 1.

**Table 1.** The optimized parameters for As(III) and As(V) analysis using DPASV.

| Parameters | Values |
|---|---|
| Deposition potential, V | -0.25 |
| Deposition time, s | 180 |
| Identity of supporting electrolyte | Hydrochloric Acid |
| Concentration of supporting electrolyte, M | 1.0 |
| Rest Time, s | 10 |
| Scan rate, V/s | 0.05 |
| Chemical reductant for the conversion of As(V) to As( | L-cysteine |

***Validation of the DPASV method for the analysis of As(III) and As(V)***

The validation of the DPASV method for the analysis of As(III) and As(V) was examined by the evaluation of the linearity, reproducibility, LOD, LOQ, and percent recovery.

Linearity was realized by running standards of As(III) and As(V) from 0 to 100 ppb, and the results are presented in Figure 5. The $r^2$ values ($r^2 = 0.99$) of As(III) and As(V) calibration curves show good linear fit for the regression analysis, indicating that the linearity range is suitable for both As(III) and As(V) analysis. The very good precision corresponds to the high sensitivity of the analytical method (Eurachem 1998). The analytical performance of the described voltammetric method was evaluated as relative standard deviation (RSD). The reproducibility of the peak heights of As(III) and As(V) standards (0 to 100 ppb) was recorded for 5 days. The obtained RSD values of no more than 13 % for As(III) and As(V) as shown in Tables 2 and 3 confirmed the very good precision of the method. The acceptable range of RSD is usually 0 to 20% for ppb levels (Kocourek 2012).

The LOD for As(III) and As(V) were calculated to be 2.24 and 6.96 ppb, respectively. These values are comparable to the LOD value of 3 ppb for As(III) obtained by Švancara et al. (2002).The LOQ for As(III) and As(V) were 7.49 and 23.19 ppb, respectively. Thus, the method is suitable for As(III) and total arsenic analysis since it can reliably detect As(III) and As(V) and quantify As(III) at concentration below 10 ppb, the maximum contamination limit set by the EPA.

**Table 2**. The peak heights of different concentrations of As(III) and their corresponding relative standard deviations.

| Concentration (ppb) | Peak height (µA)* | | | | | RSD (%) |
|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0.0130 | 0.0133 | 0.0103 | 0.0147 | 0.0140 | 12.68 |
| 10 | 0.0253 | 0.0270 | 0.0237 | 0.0267 | 0.0213 | 9.44 |
| 25 | 0.0537 | 0.0620 | 0.0657 | 0.0617 | 0.0587 | 7.42 |
| 50 | 0.1093 | 0.1047 | 0.1217 | 0.1237 | 0.1067 | 7.80 |
| 75 | 0.1607 | 0.1487 | 0.1637 | 0.1637 | 0.1567 | 3.96 |
| 100 | 0.1940 | 0.1937 | 0.2050 | 0.2050 | 0.1913 | 3.36 |

*the peak heights were already corrected by subtracting the peak height corresponding to the blank

**Table 3.** The peak heights of different concentrations of As (V) and their corresponding relative standard deviations.

| Concentration (ppb) | Peak height (µA)* | | | | | RSD (%) |
|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0.0067 | 0.0067 | 0.0080 | 0.0080 | 0.0080 | 9.78 |
| 10 | 0.0120 | 0.0130 | 0.0140 | 0.0130 | 0.0140 | 6.34 |
| 25 | 0.0353 | 0.0317 | 0.0337 | 0.0340 | 0.0350 | 4.25 |
| 50 | 0.0660 | 0.0627 | 0.0680 | 0.0640 | 0.0630 | 3.46 |
| 75 | 0.0927 | 0.0857 | 0.0917 | 0.0840 | 0.0940 | 4.99 |
| 100 | 0.1190 | 0.1157 | 0.1223 | 0.1140 | 0.1240 | 3.57 |

*the peak heights were corrected by subtracting the peak height corresponding to the blank

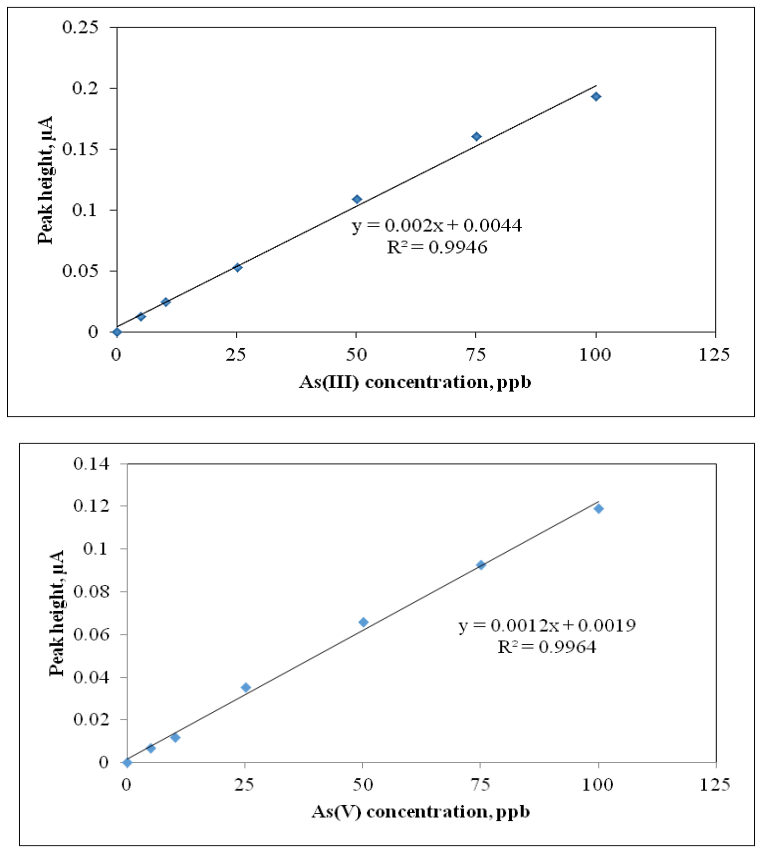



**Fig. 5**. Calibration curves of peak heights of (a) As(III) and (b) As(V) standard solutions. The As(V) concentrations were determined after reduction using cysteine. Results were obtained using the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode and Pt/Ti auxiliary electrode. DPASV parameters: electrolyte: 1 M HCl; deposition potential: -0.25 V; deposition time: 180 s; rest time: 10 s; scan rate: 0.05 V/s.

The percent recovery is a measurement of the accuracy of the method. In this study, a matrix spike was done to calculate the percent recovery. The percent recovery was analyzed by spiking 50 ppb As(III) or As(V) to a randomly selected groundwater sample. The percent recoveries for As(III) and As(V) were 91.18 ± 6.92% and 80.91 ± 5.46%, respectively. These values fall within the acceptable range since the normal percent recovery is 70 to 120% (Unsworth 2010, Midwest Laboratories, Inc. n.d.).

***Application of the method to groundwater samples and validation using ICP – OES***

Groundwater samples were collected from deep well sources in selected sites in Laguna, Philippines. These areas were selected because these were close to Mount Makiling, a dormant volcano. Arsenic content is reported to be high around volcanic areas (Hughes et al. 2011; Khan and Ho 2011). Table 4 presents the As(III) and total arsenic of groundwater samples. Most of the samples tested had undetectable concentration of As(III), the more toxic form of arsenic, however sample 4 ([As(III)] = 37.10 µg/L or 37.10 ppb) had As(III) content above 10 ppb, the maximum contamination limit set by the EPA. It is also noteworthy that samples 3 to 5 from hot spring resorts were collected at high temperature (T = 39-40$^{o}$C) during sampling and had more than 100 ppb total arsenic. The naturally high water temperature and the unusually high levels of arsenic imply that these

groundwater samples came from aquifers near Mt. Makiling. Volcanism is one of the major contributing factors for high natural arsenic levels (Hughes et al. 2011, Khan and Ho 2011).

The total arsenic content of the groundwater samples was validated using ICP – OES (Table 5). Based on the t test, the two methods were not significantly different. The statistical analysis confirms that analyses using DPASV gave accurate results.

**Table 4.** Total arsenic content of selected Laguna field water samples using DPASV. The equipment used is the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode and Pt/Ti auxiliary electrode. DPASV parameters: electrolyte: 1 M HCl; deposition potential: -0.25 V; deposition time: 180 s; rest time: 10 s; scan rate: 0.05 V/s. The measurement was done in three replicates with 3 readings per replicate.

| Sample | As(III)* (ppb) using DPASV | Total arsenic* (ppb ) using DPASV |
|---|---|---|
| 1 | nd | 37.37 |
| 2 | nd | 37.95 |
| 3 | 3.62 | 333.02 |
| 4 | 37.10 | 113.56 |
| 5 | nd | 103.14 |
| 6 | nd | 35.23 |
| 7 | nd | 32.66 |

* nd = not detected; LOD = 2.24 ppb for As(III) and 6.96 for As(V)
\+ LOD using DPASV and ICP – OES are 6.96 and 10.10 ppb As(V) , respectively

The equipment used is the eDAQ potentiostat equipped with Au electrode as working electrode, Ag/AgCl reference electrode and Pt/Ti auxiliary electrode. ICP-OES analysis was carried out using Shimadzu ICPE-9000with the following parameters: ignition mode: normal, view direction: axial, radio frequency power: 1.20 kW, plasma gas flow rate: 10.00 L/min, auxiliary gas flow rate: 0.60 L/min, carrier gas flow rate: 0.70 L/min, exposure time: 15 s, condition: wide range, solvent rinse: 30 s, sample rinse: 60 s. Each analysis was done in triplicates with 3 readings per replicate.

**Table 5.** Total arsenic content of selected Laguna groundwater samples using ICP-OES and DPASV. DPASV analysis was conducted using optimized parameters.

| Sample | Total arsenic (ppb) | | t test p-values at α=0.05* |
|---|---|---|---|
| | ICP-OES+ | DPASV+ | |
| 1 | 37.73 | 37.37 | 0.9567 |
| 2 | 42.98 | 37.95 | 0.3379 |
| 3 | 302.26 | 333.02 | 0.4720 |
| 4 | 120.94 | 113.56 | 0.1603 |
| 5 | 120.63 | 103.14 | 0.6223 |
| 6 | 31.70 | 35.23 | 0.4367 |
| 7 | 30.94 | 32.66 | 0.7417 |

*the results are significantly different if the p value is less than 0.05.

## CONCLUSION

A differential pulse anodic stripping voltammetry (DPASV) method using gold electrode as working electrode, Pt/Ti rod as auxiliary electrode, and Ag/AgCl electrode as reference electrode successfully analyzed inorganic arsenic at trace levels. The main advantages of the developed method over other analytical methods for arsenic analysis are low detection limit, low maintenance cost, high selectivity, and high sensitivity. Since the maximum contamination limit of arsenic in drinking water is 10 ppb, this developed method is an essential and useful analytical method because its detection limit for As(III) and As(V) are 2.24 and 6.96 ppb, respectively.

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