

Chapter 2

Experimental

2.1 Standards and chemicals

- 2.1.1 Arsenous acid standard (As(III), As₂O₃ 99%, Aldrich, USA)
- 2.1.2 Arsenic(V) oxide hydrate standard (As(V), As₂O₅ 99.99%, Aldrich, USA)
- 2.1.3 Hydrochloric acid (HCl 37%, AR grade, Merck, Germany)
- 2.1.4 Nitric acid (HNO₃ 69%, AR grade, Merck, Germany)
- 2.1.5 Sodium borohydride (NaBH₄ 97%, AR grade, Fluka, Switzerland)
- 2.1.6 Sodium hydroxide (NaOH 99%, AR grade, Merck, Germany)
- 2.1.7 Potassium iodide (KI 99%, AR grade, Merck, Germany)
- 2.1.8 Deionized water (From laboratory by Maxima, ELGA, England)

2.2 Instrumentation and apparatus

- 2.2.1 Inductively coupled plasma optical emission spectrometer (ICP-OES, Model Optima 4300 DV, Perkin-Elmer, USA)
- 2.2.2 Hydride generator (HGV-1, Shimadzu, Japan)
- 2.2.3 Argon gas (Ar 99.999%, Ultra High Pure grade, TIG, Thailand)
- 2.2.4 Nitrogen gas (N₂ 99.99%, High Pure grade, TIG, Thailand)
- 2.2.5 Digital balance (Mettler AE 200, USA)
- 2.2.6 Microliter pipette and Tips (Eppendorf, Germany)
- 2.2.7 Volumetric cylinder 10, 50, 100 and 500 ml (Pyrex, USA)
- 2.2.8 Volumetric flask 25, 50, 100 and 500 ml (Pyrex, USA)
- 2.2.9 Beaker 25, 50, 100 and 250 ml (Pyrex, USA)
- 2.2.10 Polyethylene bottle 500 ml (Nalgene, England)
- 2.2.11 Polyethylene tank 5000 ml (Nalgene, England)

2.3 Methodology

A Perkin-Elmer Model 4300 DV inductively coupled plasma optical emission spectrometer with axial plasma view equipped with a Shimadzu Model HGV-1 continuous flow hydride generator was used in this experiment. The reagents and instrumental conditions used for this analysis are listed in Table 3. The instrumental conditions of hydride generation-inductively coupled plasma optical emission spectrometer (HG-ICP-OES) were optimized in order to yield an optimal signal/noise ratio. The investigation of this method consisted of: the standard and reagents preparation, instrumental set up, optimization of HG-ICP-OES conditions, linear dynamic range, detection limit, accuracy, and the finally this developed method will be applied to determine inorganic As species in drinking water samples.

2.3.1 Preparation of standard and reagent solutions

All chemical used in this experiments are analytical-reagent grade (AR grade). The working standard $100 \mu\text{g L}^{-1}$ of As(III) and $100 \mu\text{g L}^{-1}$ of As(V) were prepared daily with deionized (DI) water. In addition 4 mol L^{-1} HCl and 0.4% (w/v) of NaBH_4 in 0.6% (w/v) of NaOH for hydride generation and 10% KI were prepared for the studied inorganic As species.

2.3.1.1 Preparation of working As(III) and As(V) standard solutions

The stock standard solutions in concentrations of 1000 mg L^{-1} of As(III) and As(V) were prepared as follow: As(III) stock standard solution was prepared by weigh 0.1320 g of As_2O_3 and dissolved with 1 mol L^{-1} NaOH and then the pH adjusted with conc. HNO_3 and the volume made to 250 ml with DI water. As(V) stock standard solution was prepared by weigh 0.1654 g of As_2O_5 and then the volume made to 250 ml with DI water.

The standard working solutions $100 \mu\text{g L}^{-1}$ of As(III) and As(V) can be prepared by diluting the stock standard solutions above.

2.3.1.2 Preparation of NaBH_4 solution

The reductant solution of NaBH_4 0.4 % (w/v) used was prepared by weigh 1.25 g of NaBH_4 and 1 g of NaOH then dissolving with DI water to a final volume of 250 ml. This reducing reagent solution was prepared fresh daily.

2.3.1.3 Preparation of HCl solution

The acid medium solution of 4 mol L^{-1} of HCl was prepared by pipette 83.33 mL of conc.HCl into 250 ml volumetric flask and made up the volume with deionized water.

2.3.1.4 Preparation of KI solution

The pre-reductant solution of 10% (w/v) KI used was prepared as follows: by weigh 10 g of KI dissolving with deionized water then made up the made volume to 100 ml. This KI solution was prepared fresh daily.

2.3.2 Instrumental setup

The HG-ICP-OES system set up is shown in Figure 4.

2.3.2.1 Hydride generation system

The continuous hydride generation system consisted of: a three-channel peristaltic pump (4300DV ICP-OES instrumental) with three pump tubing (Trgon, 6.5 mm i.d. for sample and Tygon, 3.5 mm i.d. for NaBH_4 and HCl) a mixing coil and gas-liquid separator were used.

The hydride generation conditions used were optimized. For the first study the flow rates of sample selected was 3.6 mL min^{-1} , HCl and NaBH_4 both 1.2 mL min^{-1} . Arsenic in the sample was reduced to arsine or hydride gas by using the reductant in an acid medium. The three solutions were pumped with a three channel peristaltic pump into the manifold. After mixing the solutions were transported with argon carrier gas into the mixing coil, and passed to the gas/liquid separator for separating arsine gas into the hydride connector of the

ICP-OES system for analysis. The details and description of the hydride generation system and conditions are shown in Table 3.

2.3.2.2 ICP-OES system

A Perkin-Elmer Model 4300 DV inductively coupled plasma optical emission spectrometer with radial plasma view equipped with a Shimadzu Model HGV-1 continuous flow hydride generator was used in this experiment. The detailed descriptions of the ICP-OES instrument system and conditions are shown in Table 3. The data of arsenic emission signals were relayed to a printer. The data acquisition was realized with specific software, Winlab32. The integration peaks were achieved by area measurement.

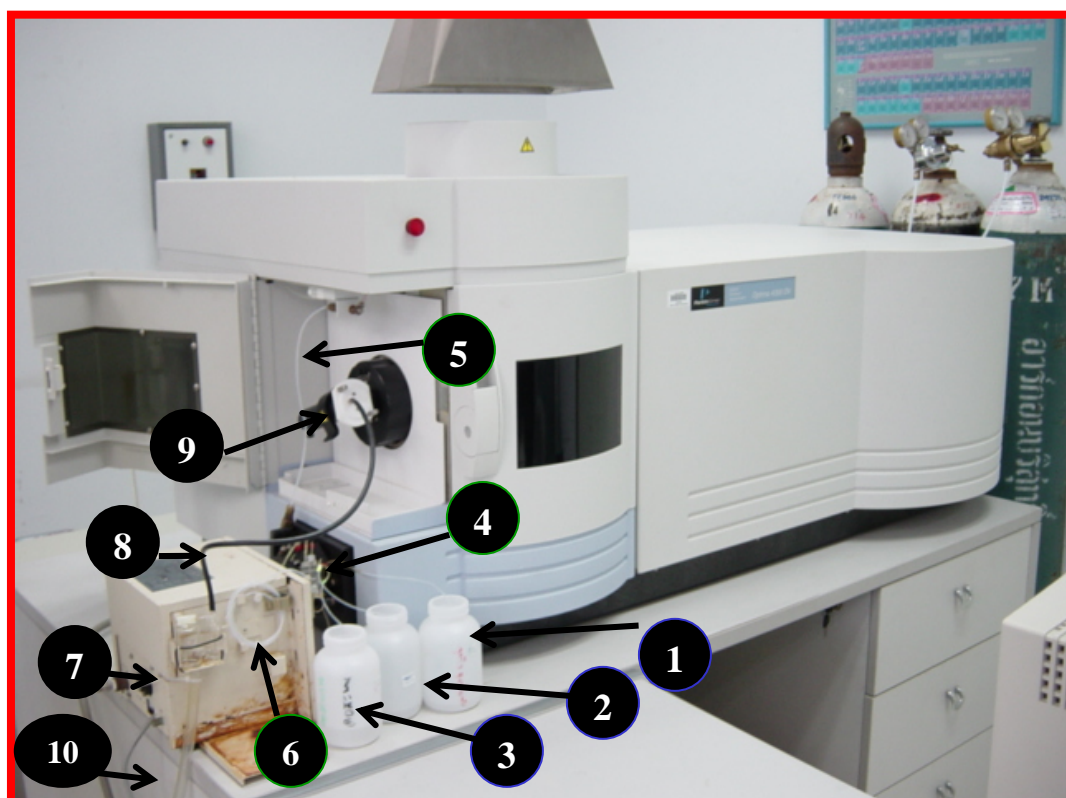


Figure 4 Instrumental setup of HG-ICP-OES system was used in these experiments consisted of; 1. Sample, 2. HCl, 3. NaBH₄, 4. Peristaltic pump, 5. Ar carrier gas, 6. Mixing coil, 7. Gas/liquid separator, 8. Hydride gas, 9. Hydride connector, and 10. Waste.

Table 3 Experimental conditions for the HG-ICP-OES**Inductively coupled plasma optical emission spectrometer condition**

Wavelength ¹	193.7 nm
Measurements integration time ²	
Maximum	10 second
Minimum	5 second
RF power ³	1.4 kW.
Argon gas flow rates	
Nebulizer (Carrier gas) ⁴	0.8 L min ⁻¹
Plasma ⁵	15 L min ⁻¹
Auxiliary ⁶	0.5 L min ⁻¹
Generator	40 MHz
Delay time	30 second
Replicates	5
Plasma viewing	axial

Hydride generation condition

Pumping rate ⁷	0.8 mL min ⁻¹
Sample flow rate ⁸	3.6 mL min ⁻¹
NaBH ₄ and HCl flow rate ⁹	1.2 mL min ⁻¹
NaBH ₄ Concentration ¹⁰	0.4 % (w/v)
HCl Concentration ¹¹	4 mol L ⁻¹

¹⁻¹¹ These parameters were changed or optimized during this study

2.3.3 Optimization of the HG-ICP-OES conditions for arsenic analysis

The optimum conditions of continuous flow hydride combined with an inductively coupled plasma optical emission spectrometer system used in this study are shown in Table 3. The optimum conditions of the ICP-OES system used were based on the following parameters; wavelength, integration time, RF power, plasma argon gas flow rate, auxiliary argon gas flow rate. The optimum conditions of the continuous flow HG system used in this studied were; NaBH₄ and HCl concentration, Ar carrier gas flow rate, pumping rate, sample flow rate, NaBH₄ and HCl flow rate, and KI concentration and time.

2.3.3.1 Wavelength

The optimum wavelengths of the As(III) emission signal was studied at various wavelengths of 189.0, 193.7, 197.3 and 288.8 nm as following of ICP-OES manual, with a 100 µg L⁻¹ As(III) standard solution. The standard solution was analyzed by the HG-ICP-OES system under the conditions in Table 3. The optimum wavelength was selected as the one with the highest emission intensity.

2.3.3.2 Integration time

The optimization of integration time was investigated by using the same standard solution in 2.3.3.1. The standard solution was analyzed by the HG-ICP-OES system under the conditions in Table 3 with wavelength as found from 2.3.3.1. The integration time was studied by varying the time in the range of 1-5, 5-10, 10-20 and 20-50 seconds. The highest detectable emission intensity selected was the optimum of the integration time observed.

2.3.3.3 RF power

The optimization of RF power was performed by using the same standard solution in 2.3.3.1. The standard solution was analyzed by the HG-ICP-OES system under the conditions in Table 3 and value of the results from 2.3.3.1-2.3.3.2. The RF power was tested at 1, 1.2, 1.3, 1.4 and 1.5 kW, and then the optimum of the RF power was selected as the highest emission intensity obtained.

2.3.3.4 Plasma gas flow rate

The optimization of the flow rate of the plasma gas was studied by varying flows of 15, 16, 17, 18, 19 and 20 L min⁻¹. Other parameters were set as the conditions described in Table 3 and from the results of experiments 2.3.3.1-2.3.3.3. The optimum plasma gas flow rate was selected as the one giving the highest emission intensity.

2.3.3.5 Carrier gas flow rate

The carrier gas flow rates was studied by varying flows from 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 L min⁻¹. The optimum of carrier gas flow rate was obtained from are giving the highest emission intensity. The same parameters were set as the conditions given in Table 3 and from the results of 2.3.3.1-2.3.3.4.

2.3.3.6 Auxiliary gas flow rate

The previous parameters were set according to conditions given follows in Table 3 and from the results from 2.3.3.1-2.3.3.5. The optimization of the flow rate of the auxiliary gas was studied by varying the gas flow rate from 0.10 0.2, 0.3, 0.4, 0.5 and 0.6 L min⁻¹. The optimum flow rate of the auxiliary gas was selected by considering the best emission signal of As(III).

2.3.3.7 NaBH₄ concentration

The emission signal of the As(III) standard was studied using various NaBH₄ concentrations of 0.1, 0.2, 0.3, 0.4, 0.8 and 1% (w/v). The described conditions in Table 3 and the results from 2.3.3.1-2.3.3.6. The optimum concentration of NaBH₄ was selected that with the highest emission intensity.

2.3.3.8 HCl concentration

The optimum concentration of HCl was studied by varying the concentration from 0.5 to 4 mol L⁻¹. The highest detectable emission signal was selected as the optimum for the HCl concentration. The set parameters those obtained from the result of 2.3.3.1-2.3.3.7.

2.3.3.9 Pumping rate

The emission signal of the As(III) standard was studied using various pumping rates of 0.3, 0.5, 0.8, 1 and 1.5 mL min⁻¹. The HG-ICP-OES system was used under conditions given in Table 3 and from the results of 2.3.3.1-2.3.3.8. The highest detectable emission signal was selected as the optimum pumping rate.

2.3.3.10 Sample flow rate

The optimization of the flow rate of the sample was varied from 0.6, 1.2, 3.6, 4 and 6 mL min⁻¹ using the same parameters as described in Table 3 and from the results from 2.3.3.1-2.3.3.9. The highest emission intensity was selected as the optimum sample flow rate.

2.3.3.11 NaBH₄ and HCl flow rate

The optimization of the flow rate of NaBH₄ and HCl was studied using flow rates of 0.2 to 2 mL min⁻¹ the same conditions as described in Table 3 and plus the results from 2.3.3.1-2.3.3.10. The highest detectable emission signal was selected as the optimum for the NaBH₄ and HCl flow rates.

2.3.3.12 KI concentration

The determination of total As concentration was performed after a complete reduction of As(V) to As(III) with KI. The emission signal of total As with mixed standards of 4 µg L⁻¹ As(III) and 4 µg L⁻¹ As(V) standard was studied using various KI concentrations of 5, 10, 20, 40 and 60%. The same parameters were set as the conditions obtained as follows in Table 3 and from the results of 2.3.3.1-2.3.3.12. The best emission signal obtained was selected as the optimum concentration of KI.

2.3.3.13 KI time

The optimum time to reduce As(V) to As(III) was studied using various KI times of 5, 10, 20, and 30 min. The same parameters were in

Table 3 and from the results of 2.3.3.1-2.3.3.12. The highest emission signal found was selected as the optimum time of KI.

2.3.4 Linear dynamic range

The dynamic range can be specified as the concentration range or analytical signal range over which the analytical curve is linear or the calibration slope is constant. It is usually defined at the lower end by the detection limit and at the upper end by an analyte concentration where the analytical signal deviates a specific relative amount (e.g., 5%) from the extrapolated linear portion of the curve or where the slope deviates a specific relative amount from the slope in the linear portion. Non linearity can be inherent in the technique or due to the matrix of the standards, non ideal instrumental performance, instrumental distortion, or incorrect utilization of the instrument. A linear calibration curve is usually preferred because it is easier to detect an abnormality and because it is easier to work with mathematically. A large dynamic range is preferred because a wide range of analyte concentrations can be used without sample dilution. A nonlinear calibration curve can be used as long as enough standards are measured to establish the calibration function (Ingle and Crouch, 1988)

The linearity of the calibration curve was established using three series of total As, As(III) and As(V) standard solutions with concentrations of 1, 5, 10, 25, 50, 75 and 100 $\mu\text{g L}^{-1}$. The linear dynamic range was obtained by plotting the detector response (emission intensity-Y axis) *versus* the concentration (X-axis). Each slope of the calibration curve were evaluated as a linear equation.

2.3.5 Limit of detection and limit of quantitation

The limit of detection (LOD) was defined as the smallest concentration which could be measured with a specified degree of certainty (IUPAC Definition) and is defined arbitrarily as $3S_B$ (Currie, 1999 and Taylor, 1987).

The lower level where measurements become quantitatively meaningful has been called the limit of quantitation (LOQ) (IUPAC Definition) and is defined arbitrarily as $LOQ = 10S_B$ (Currie, 1999 and Taylor, 1987). In this work, the LOD and LOQ were studied. The limit of detection and limit of quantitation

were established by using series of total As and As(III) standard solutions with concentrations of 1, 5, 10, 25, 50, 75 and 100 $\mu\text{g L}^{-1}$ and 10 replications deionized water as a blank. The calibration curve was plotted and the mean value of the blank responses (X_B) and the standard deviation (S_B) were calculated. Each slope of the calibration curve were evaluated as a linear equation.

The probability that the smallest discernible analytical signal (X_L) can be measured is

$$X_L = X_B + kS_B \quad (1)$$

Where k is a numerical factor chosen in accordance with the confidence level desired. C_L , the limit of the detection concentration, is a function of X_L , *i.e.*

$$C_L = (X_L - X_B) / m \quad (2)$$

Where m is the analytical sensitivity. Because the average blank reading, X_B , is not always 0, the signal must be corrected with the background, By substituting Equation 1 in to Equation 2.

$$C_L = kS_B / m$$

Where C_L = Limit of detection concentration value (IUPAC Definition)

$k = 3$ allows a confidence level of 99.86%

S_B = the standard deviation of blank

m = the analytical sensitivity

2.3.6 Accuracy

The accuracy indicates how close the measured analyte concentration in the sample is normally to the actual value and is expressed as the relative percent error. The accuracy depends on the analyte concentration, precision and interference effects.

The accuracy of this experiment was conducted by using recovery. The recovery is best evaluated by measurement of known composition and spikes or added to the sample. The recovery is often stated as the percentage measured with respect to what was added. Complete recovery (100%) is of course the ultimate goal (Taylor, 1987). The % recovery can be evaluate by spiking of 1, 5, 10, 25, 50, 75 and 100 $\mu\text{g L}^{-1}$ of As(III) and As(V) in to a blank and samples respectively.

2.4 Samples analysis

This investigation shows that this technique can be applied to drinking water samples for inorganic As species. The drinking water samples were purchased from the supermarket and the detail of these are shown in Table 4 and Figure 5.

Table 4 Drinking water samples for total As, As(III) and As(V) analysis using HG-ICP-OES

Sample No.	Sampling date
1	23/09/05
2	23/09/05
3	23/09/05
4	23/09/05
5	23/09/05



Figure 5 Drinking water samples used in these experiment

2.4.1 Determination of inorganic As in drinking water

Inorganic As in drinking water was determined by using the optimum conditions established in this study. Due to the very low concentration of arsenic in drinking water in this study the spiking technique must be applied to measure inorganic As. In addition, % recovery was also studied to check the accuracy of this method.