Applied Chemistry Project

Project title	Colorimetric Paper-based Sensor of As(III)		
Student names	Miss Yinxi Zhu	ID	6033844523
Program	Bachelor of Science in Applied Chemistry		
Academic year	2020		

Faculty of Science, Chulalongkorn University

Colorimetric Paper-based Sensor of As(III)

by

Miss Yinxi Zhu

In Partial Fulfillment for the Degree of Bachelor of Science

Program in Applied Chemistry (International Program)

Department of Chemistry, Faculty of Science

Chulalongkorn University

Academic Year 2020

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By Miss Yinxi Zhu

Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science Program in Applied Chemistry (International Program)

Examination committees

1. Assistance Professor Charoenkwan Kraiya, Ph.D.	Committee
2. Associate Professor Nattaya Ngamrojanavanich, Ph.D.	Committee
3. Professor Orawon Chailapakul, Ph.D.	Advisor

Endorsed and approved by the Head of Department of Chemistry

....	....
(Professor Orawon Chailapakul, Ph.D.)	(Associate Professor Voravee Hoven, Ph.D.)
Advisor	Head of Department of Chemistry
	28 December 2020
	Date

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Student Name Miss Yinxi Zhu Student ID 6033844523

Advisor Name Professor Orawon Chailapakul, Ph.D.

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Abstract

The novel paper-based colorimetric device using the hydride generation and Au(III) was developed for the determination of As(III) using a smartphone. The analysis of As(III) is based on arsine gas from hydride generation oxidized Au(III) to Au(0). In the presence of As(III), the distinctive color change of the Au(III) from colorless to violet was visualized by the naked eyes within 3 min. The Au-enhanced Au particles strategy as the seeding method was performed to improve the sensitivity of the developed device. For the enhancing step, Au(0) was enlarged by the catalytic reaction of $K[AuCl_4]$ and ascorbic acid. Under optimal conditions, a good linear relationship between the logarithm of arsenic concentration and the average mean color intensity was found in the arsenic range of 5 - 1000 ppm. The limit of detection (LOD) was observed as low as 1 ppm (3SD/slope). Furthermore, the developed device presents high selectivity against common heavy metals interferences. The proposed device shows excellent benefits, for example, low cost, ease of use, less time requirement and high selectivity.

Keywords: paper-based analytical device, arsenic determination, colorimetric detection, hydride generation

Acknowledgment

First of all, I would like to express my special thanks of gratitude to my advisor, Professor Dr. Orawon Chailapakul, who allowed me to do this project in the electroanalytical field. Her guidance, understanding, and scrutiny have encouraged me to make progress in my project. It has been a great pleasure and honor to have her as my advisor.

My deepest gratitude goes to Dr. Kingkan Pungjunun for guiding me all the time. Without her help, I do not think I can pull this project through. I am also thankful to Chawin Srisomwat for inspiring me with a new idea for achieving my project better. Besides, I also want to extend my thanks to Dr. Abdulhadee Yakoh, Suchanat Boonkaew, Whichuta Jesadabundit, Pannaporn Pusomjit, Sarida Naorungroj, Dr. Atchara Lomae and Thanathip Kosawatphat for their motivation and sincere help during my project. In fact, all lab members provide their help and support; I thank them wholeheartedly.

Last but not least, I appreciate my parents and grandparents' love and support unconditionally. I am the luckiest to have them be my back.

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Chapter 1

Introduction

1.1 Introduction to the research problem and significance

Arsenic is classified as a metalloid element with the symbol As. It has naturally existed in the earth's crust for about 1 – 1.8 mg/kg[1]. However, As is considered a heavy metal due to its toxicity to humans and organisms. Typically, the toxicity of As depends on its forms. It is well-established fact that arsenite (As(III)) is more toxic than arsenate (As(V)). In addition, inorganic As is more toxic than organic As[2]. The major As contamination can be caused through the advance in industrial (such as large-scale mining, smelting and production manufacturing). At each stage of the industrial As process, its residue could contaminate the environment through the exhaust, slag, and final product application. Besides the environmental impact, a high level of As significantly affect human health. As enters the human body through the respiratory tract, digestive tract and skin contact. The high level of As accumulation in a human organ such as liver, kidney, lung, spleen, uterus, and bones can cause carcinogenic and skin cancer. For controlling the As contamination, the world health organization (WHO) has established the current recommended limit of As in drinking water, in which it should have existed less than 10 ppb or 50 ppb. However, more than 100 million people worldwide, particularly in developing countries, are still at risk with the critical contamination of more than 50 ppb[4]. Therefore, the determination of As has attracted increasing attention, and the method has been continuously developed.

There is a numerously conventional method for the determination of As such as spectrophotometry[5-6], atomic absorption spectrometry (AAS)[7], inductively coupled plasma atomic emission spectroscopy (ICP-AES)[8] and atomic fluorescence spectrometry (AFS)[9]. However, these techniques have some limitations, including bulky instrumentation, large sample volume, complicated technique and high cost. Due to the merit of simple operation and easy interpretation, the colorimetric assay has been employed in a broad prospect for the development of analytical determination. This method is considered as one of the optical sensors relying on the color change from chemical reactions such as redox reactions[10], precipitation[11], and surface plasmon resonance[12]. The color change is generally captured by an imaging device (such as a smartphone or camera-included device). Then, the color intensity, which is proportional to the analyte concentration, is obtained from image software such as ImageJ software. Consequently, the colorimetric method is the most attractive technique for semi-quantitative analysis.

Nowadays, a colorimetric determination has been miniaturized couple with paper-based analytical devices (PADs) to avoid using sophisticated equipment and large reagent consumption. Therefore, the paper-based device has come into the perspective of researchers because of its affordable material, low cost, recyclability and environmental degradation. Due to the hydrophilicity and capillary phenomenon of the paper itself, the PADs offer the advantages of design flexibility of the flow system.

Herein, the paper-based analytical device was developed for the determination of As(III) utilizing Au(0) particles as a remarkable colorimetric agent. Upon the addition of As solution into the device, AsH_3 (arsine gas) was generated by a reduction reaction between As(III) and $NaBH_4$ in acidic conditions (known as hydride generation). Then, Au(III) was used to oxidize the obtained arsine gas, resulting in the color change from colorless to violet. However, the colorimetric assay still suffers from its low sensitivity. Therefore, the Au-enhanced Au particles was employed in this work to improve the sensitivity. In this step, ascorbic acid was utilized to reduce the other Au(III) for enlarged Au(0) particles. The attained Au(0) particles from the reduction of ascorbic acid gradually grown onto the Au(0) to enhance the sensitivity. To facilitate the application of this sensor for on-site testing, a smartphone was used to monitor the colorimetric responses in the detection platform. The simple, disposable and portable device was demonstrated in this work.

1.2 Research objectives

- To develop a simple and portable colorimetric paper-based analytical device for the determination of As(III) using a smartphone

- To enhance the sensitivity of the developed device using Au-enhanced Au particles method

1.3 Literature researches

There are several methods for As determination, including spectrometric method and colorimetric method as follows:

1.3.1 Spectrophotometric method using silver nitrate as a color reagent

In 2018, Pena-Pereir et al. developed the spectrophotometric method for As(III) determination in the water using $AgNO_3$ as a color reagent. KBH_4 (or $NaBH_4$) was employed to produce the new ecological hydrogen in the acidic solution and reduce the inorganic As in the water to arsine gas. This study selected nitric acid, $AgNO_3$, polyvinyl alcohol, and ethanol solution as absorbing liquid. For the detection mechanism, arsine gas was used to reduce the silver ions in the absorption solution to the single colloidal silver, causing the change of color from the colorless solution to the yellow solution. This yellow solution presented the maximum absorption at 400 nm with a symmetrical peak shape. The color intensity was proportional to the amount of As(III) with the detection limit as low as 10.2 ppm[5].

1.3.2 Spectrophotometric method using silver diethyldithiocarbamate as a color reagent

In 2000, Arbab-Zavar et al. reported the spectrophotometric determination of inorganic As(III) by silver diethyldithiocarbamate as an absorbing complex. In this study, zinc was used to produce new ecological hydrogen under acidic conditions. The attained ecological hydrogen from the reduction reaction in potassium iodide and tin chloride can reduce As(III) to arsine gas. After that, arsine gas was absorbed with silver diethyldithiocarbamate in chloroform to generate red colloidal silver. The absorbance of the colloidal silver was measured at a wavelength of 525 nm. The detection limit of 0.5 μg and a concentration detection limit of 0.05 μg/ml were obtained using a 10 mL sample volume[6].

1.3.3 Atomic absorption spectrometry (AAS) method

Atomic absorption spectrometry (AAS) method proposed by the United States Environmental Protection Agency in 2015 for trace level determination of antimony (Sb) and total As at 1 ppb to 400 ppb, respectively, in the surroundings. KBH_4 or $NaBH_4$ in an acid solution was chosen for new ecological hydrogen generation. Its hydrogen product can reduce inorganic As(III) in the water sample to AsH_3 (arsine gas). Arsine gas was loaded into a quartz tube with Ar gas and heated the quartz tube to 1000 °c by electric heating. At this temperature, arsine gas was decomposed to form As atom vapor, which absorbed the characteristic electromagnetic radiation from As light source. This proposed system exhibited a detection limit of 1.0 ppb for As(III) [7].

1.3.4 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP - AES) detection

High-performance liquid chromatography coupled to ICP-AES detection for As(III) and As(V) was reported by Chausseau et al. in 2000. Plasma emission spectroscopy could simultaneously determine the content of As in the sample. The plasma torch could reach a high temperature of 600 to 8000 °C. Under the high temperature, the complex of total As could be atomized to As atoms and the emitting intensity of the characteristic spectrum relating to total As concentration in the samples. The detection limits of 0.15×10^{-2} and 0.19×10^{-2} mM for As(III) and As(V) were obtained[8], respectively.

1.3.5 Atomic Fluorescence Spectrometry (AFS) method

Jing Zhou et al. reported that AFS was a suitable alternative method combined with cadmium ions (Cd^{2+}) assisted photochemical vapor generation (PVG) for the determination of the total As. PVG-AFS technique is performed to generate volatile species containing the target element and then its gas is detected by the AFS method. PVG efficiency was discussed using a

20 ppb As(III)/As(V) solution added with 20 ppm Cd^{2+} and 30% (*v*/v) acetic acid with UV irradiation for 60 s. This system provided the detection limits for the total As of 0.05 ppb[9].

Among previous literature, the silver nitrate spectrophotometric method was attractive for the determination of As(III) in water and wastewater because of its fast and sensitive features. However, this method still requires large amounts of reagents and samples. Moreover, this technique has some drawbacks, including high cost, bulky instrument, and complicated technique. Thus, the paper-based sensor was developed for As(III) determination with the high performance in terms of low-cost, disposable and portable device to overcome the limitation of previous works. In addition, less volume of reagents and samples are required to apply in the paper-based sensor.

1.3.6 Silver nitrate reaction in the paper-based sensor

The paper-based analytical device (PAD) was first reported by Pena-Pereir et al[5]. for the detection of the total As relying on the reaction between Ag(I) and arsine gas. Ag(I) acted as a color reagent for arsine gas in the paper-based sensor. The total As in solution was reduced to arsine gas by $NaBH_4$ in acidic conditions. The chemical reaction between Ag(I) and the analyte derivative results in the change of color from colorless to yellow, as the following:

$$H_3AsO_3 + 2BH_4^- + 2H^+ \rightarrow 2AsH_3 + 2H_3BO_3 + 3H_2 \qquad (1)$$

$$AsH_3 + 6AgNO_3 \rightarrow AsAg_3{\cdot}3AgNO_3 + 3HNO_3 \qquad (2)$$

$$AsAg_3{\cdot}3AgNO_3 + 3H_2O \rightarrow H_3AsO_3 + 6Ag^0 + 3HNO_3 \qquad (3)$$

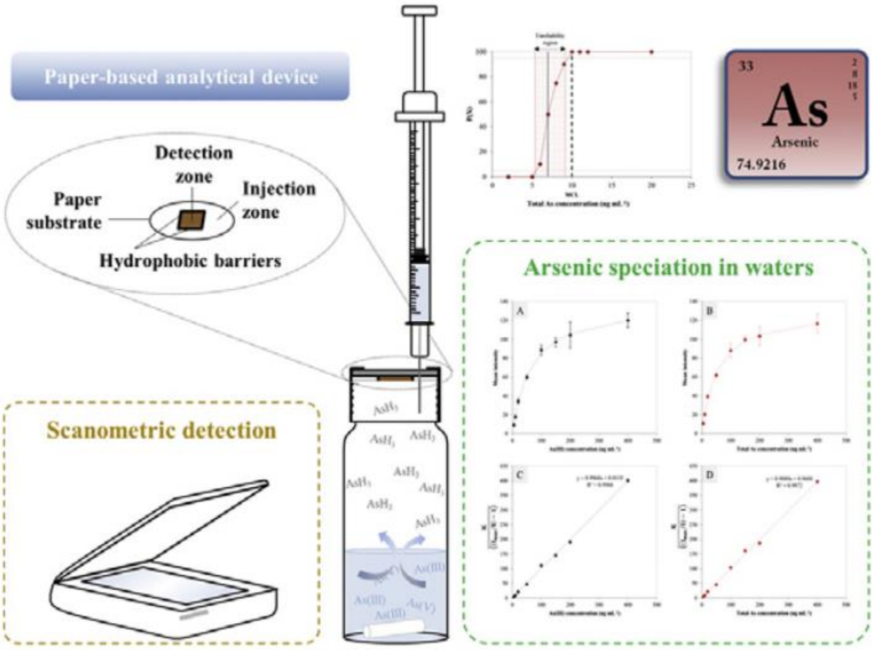

Figure 1.1 Schematic representation of the developed system for the determination of the total As[6].

The intensity of color-related with the concentration of total As can be analyzed by the Image J program. This method showed the limit of detection of 1.1 ppb. However, this method still requires a large volume of reagents and samples. Therefore, we would like to develop this system on PADs for the determination of As(III).

Moreover, the colorimetric determination of As(III) on PADs still suffers due to the low detection limit. To improve the sensitivity, the Au-enhanced Au particles was introduced in this work.

1.3.7 Au-enhanced Au particles

Razo et al. reported the lateral flow immunoassay (LIFA) for rapid field-based control of bacterial targets. The lateral flow test strips were performed using gold nanoparticles size of 17.4 nm as a label. However, the high limit of detection (LOD) is a significant drawback for this proposed method. Reducing the LOD is a critical task in developing LFIA test systems. Therefore, the Au-enhanced Au particles was operated to overcome this limitation. In this work, the solution of hydroxylamine was used to reduce Au(III) to Au(0), which will further coat on the surface of AuNPs. The results presented that the gold enlargement in the test zone of a lateral flow test strip could enhance the color signal at a low concentration of target analytes[13].

Chapter 2

Experiment

2.1 Chemicals

Table 2.1 Chemical list

Name of chemical	**Company**
Hydrochloric acid 37%	Emsure®
L (+) ascorbic Acid	Carlo Erba
Sodium borohydride	Sigma-Aldrich
Potassium tetrachloroaurate (III)	Wako
Cadmium standard solution	BDH
Lead(II) standard solution	BDH
Zinc(II) standard solution	Merck
Copper(II) standard solution	Fluka
Chromium(VI) standard solution	Merck
Silver nitrate	Poch

2.2 Instruments

2.2.1 In-house light control box with the smartphone

A smartphone is placed on top of the black box to control ambient light. The image of the paper-based device was then captured and analyzed with the ImageJ application.

2.2.2 Wax-patterned paper fabrication

Circular areas with a diameter of 0.5 cm were designed by Adobe illustrator CS4 software (Adobe System, Inc.). The wax printing method was used for the fabrication of the hydrophobic area of PADs. The blue color with an RGB value of (0/173/247) was used to be complementary to the colorimetric reaction. The process to fabricate PADs consisted of two steps: (1) printing the wax pattern on the surface of filter paper (Whatman no.1) by a commercial wax printer (Xerox Color Qube 8570, Japan) and (2) melting the wax printed paper at 165 °c for 3 minutes on a hot oven. Consequently, the printed wax can penetrate the paper and entirely create a hydrophobic barrier and hydrophilic channels.

2.3 Reagent preparation

2.3.1 Preparation of 1,000 ppm As(III)

A stock solution of 1,000 ppm As(III) was prepared by dissolving 43.34 mg $NaAsO_2$ (formula weight: 129.91) in 25 mL of MilliQ water.

Table 2.2 Volumes of stock solutions pipetted to prepare a concentration series of As(III) solution

Concentration (ppm)	Volume of 1000ppm As (μL)	Volume of MilliQ water (μL)	Total volume (μL)
750	750	250	1,000
500	500	500	1,000
250	250	750	1,000
100	100	900	1,000
50	50	950	1,000
25	25	975	1,000

10	10	990	1,000
5	5	950	1,000
1	1	999	1,000

2.3.2 Preparation of 1,000 ppm Ag(I)

$AgNO_3$ was used as a recognition element for the pattern I. 0.2 g of $AgNO_3$ (formula weight: 169.87 g/mol) was added to the plastic tube. 2 mL of MilliQ water was added to the plastic tube and mixed. The solution should be stored in the fridge at -20 °c before use.

2.3.3 Preparation of Au(III)

Au(III) was prepared by weighting $HAuCl_4$ (formula weight: 377.88 g/mol) in a plastic tube and dissolved by 2 mL MilliQ water directly.

Table 2.3 Weights of $HAuCl_4$ to prepare a concentration series of Au(III) solution

Concentration of Au(III) (ppm)	Weight of $HAuCl_4$ (mg)	Total volume (mL)
500	1.92	2
1,000	3.83	2
1,500	5.75	2
2,000	7.66	2

2.3.4 Preparation of hydrochloric acid (HCl)

HCl was prepared from 37% stock HCl solution (density 1.19kg/L) and diluted by MilliQ water into 20 ml in total volume.

Table 2.4 Volumes of stock solutions pipetted to prepare a concentration series of HCl solution

Concentration (M)	Volume of 37% stock HCl solution (mL)	Volume of Milli-Q (mL)	Total volume (mL)
7.5	12.43	7.57	20
5.0	10.29	9.71	20
2.5	4.14	15.86	20

2.3.5 Preparation of sodium borohydride ($NaBH_4$)

$NaBH_4$ was prepared by weighting $NaBH_4$ (formula weight: 37.83 g/mol) in a tube, then directly adding Milli-Q water.

Table 2.5 Preparation of $NaBH_4$

Concentration (%)	Mass of $NaBH_4$ solid (g)	Total volume (mL)
7.5	0.15	2.0
5.0	0.10	2.0
2.5	0.05	2.0

2.3.6 Preparation of 5 mM ascorbic acid (AA)

AA was weighted 0.0088 g (formula weight 176.13 g/mol) and dissolved in 10 mL of MilliQ water.

2.3.7 Au(III)-Ascorbic acid solution (Au-AA solution)

A stock solution of 1,000 ppm Au(III) was prepared as previously stated.

Table 2.6 Au(III) preparation

Concentration (ppm)	Volume of 1,000 ppm Au (μL)	Volume of MilliQ water (μL)	Total volume (μL)
5	5	995	1,000
2.5	2.5	997.5	1,000
1	1	999	1,000

500 μL of AA solution was added in Au(III) 500 μL (ratio Au: AA = 1:1) and then mixed.

2.4 Device fabrication

There are 2 different patterns fabricated for the As experiment. The pattern I was designed for preliminary study while Pattern II was developed for the investigation of the Au-enhanced Au particles. The pattern designs were created by Adobe Illustrator CC (Adobe Systems, USA). The wax pattern was printed onto Whatman No.1 filter paper using a wax printer (Xerox ColorQube 8570, Japan) and then placed in an oven at 165 °c for 3 minutes. After that, the transparent tape was sealed on the backside of the device to prevent the solution leakage.

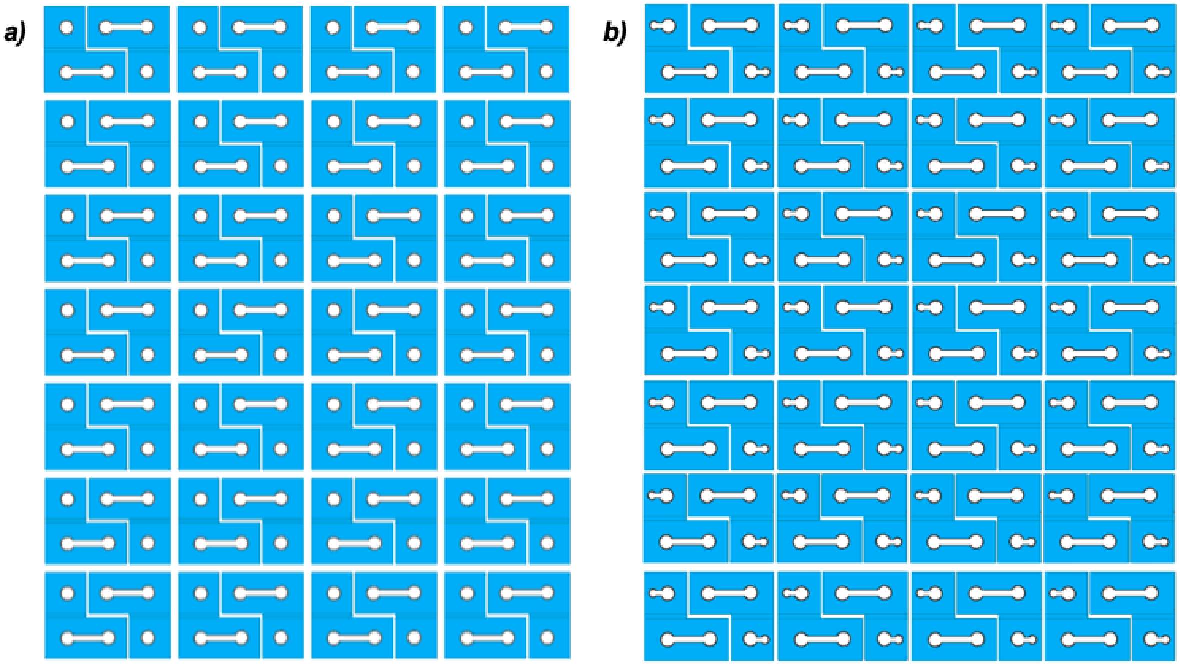

Figure 2.1 The pattern I (a) and II (b)

2.4.1 The pattern I fabrication

The pattern I was cut into 3 cm x 1.5 cm rectangle and connected with 1.5 cm x 1.5 cm square. The rectangle paper substrate, known as a sample PAD, is consisted of 2 hydrophobic barriers with a circular side of 5 mm diameter defined as the reaction area and sample inlet area (Figure 2.2a). These 2 circles were connected by the elongated channel to let the solution passing through. A circle (5 mm: diameter) existed on the detection PAD was used as a detection zone (Figure 2.2a).

Before use, 2 µL of the $AgNO_3$ was spotted in the detection zone by micropipette. Then 2 µL of $NaBH_4$ was spotted in the reaction zone of the sample PAD. The 2 µL of HCl solution was spotted in the sample inlet zone, as shown in Figure 2.2.b. After reagent spotting, the PADs were allowed to dry at room temperature for 20 minutes.

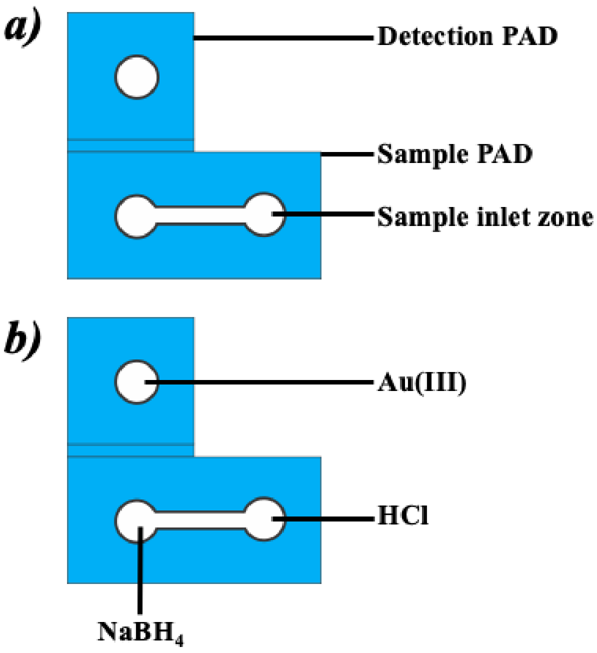

Figure 2.2 Reagent spotting on the pattern II device

The punched acrylic tape was used to connect the back of the detection PAD and the front of the sample PAD to create the space where the gas generation reaction takes place. The detection PAD was folded to the sample PAD, as shown in Figure 2.3a. Figure 2.3b illustrates the top view and the side view of the device (pattern I).

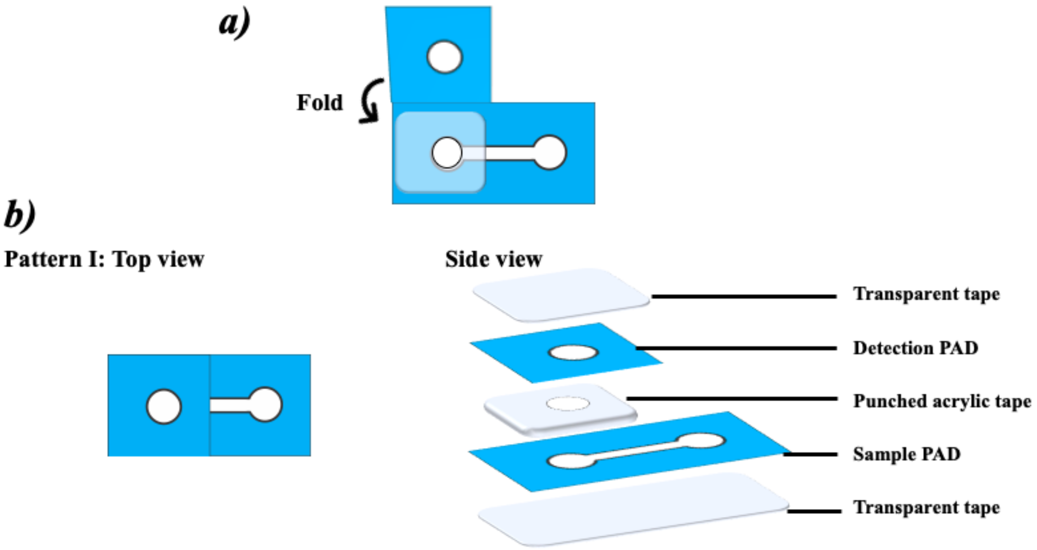

Figure 2.3 The origami pattern I design (a) with the top and side view of the device (b)

2.4.2 Pattern II fabrication

The pattern II was designed for the Au-enhanced Au particles. A similar fabrication method as in pattern I was used for this pattern. However, the circle with a 3 mm diameter was added in this pattern for the Au-AA inlet zone, as shown in Figure 2.4a.

2 μL of the Au(III) was spotted in the detection zone by pipette. Then 2 μL of $NaBH_4$ was spotted in the reaction zone of the sample PAD. Then, 2 μL of HCl solution was spotted in the sample inlet zone and allowed to dry for 20 minutes, as shown in Figure 2.4b.

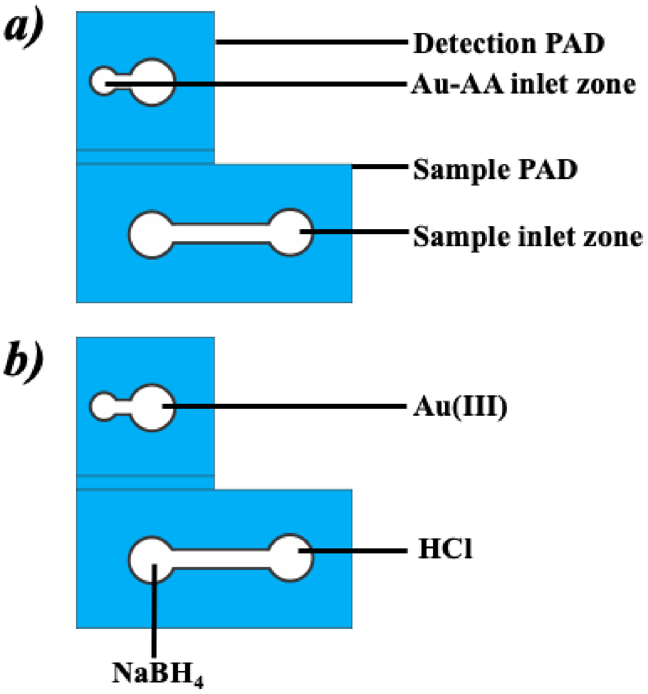

Figure 2.4 Reagent spotting on the pattern II device

After drying, the detection PAD was folded to the sample PAD and attached with the acrylic tape. The transparent tape attached to the front of the detection PAD was punched with a 3 mm diameter hole for the Au-AA inlet zone, as shown in Figure 2.5a. Figure 2.5b presents the top view and the side view of the device (pattern II).

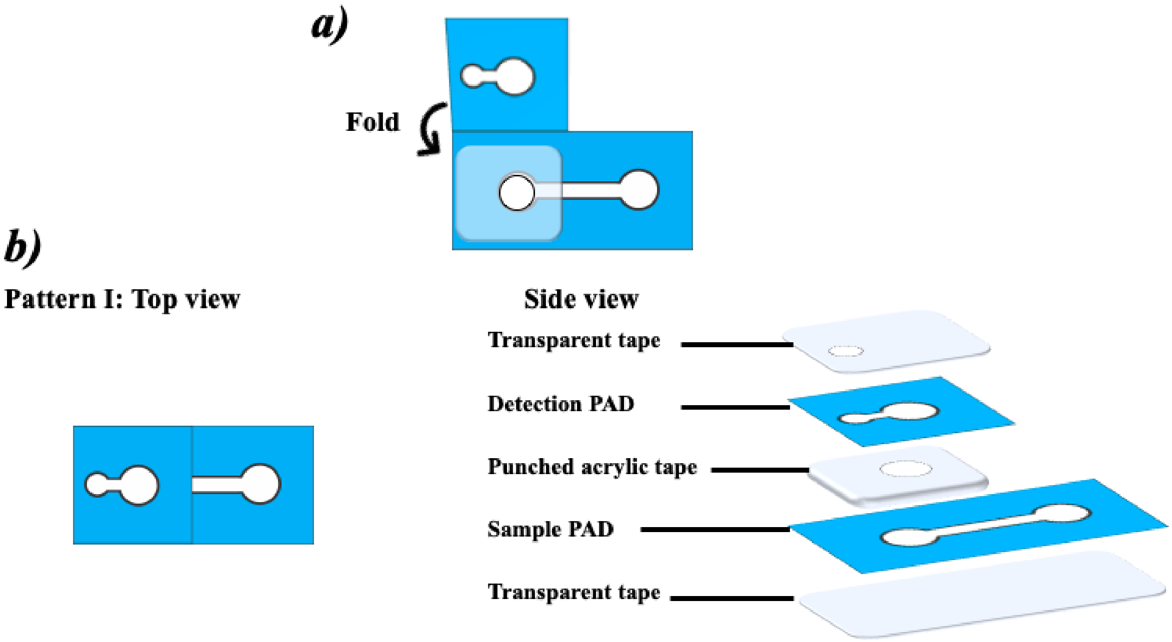

Figure 2.5 The origami pattern II design (a) with the top and side view of the device (b)

2.5 Device Operation

For the pattern I, 10 µL of As(III) sample solution was added to the sample inlet zone. After that, the device was placed into the In-house light control box. After 3 minutes, the color change image was captured using a smartphone and analyzed the color density by Image J software.

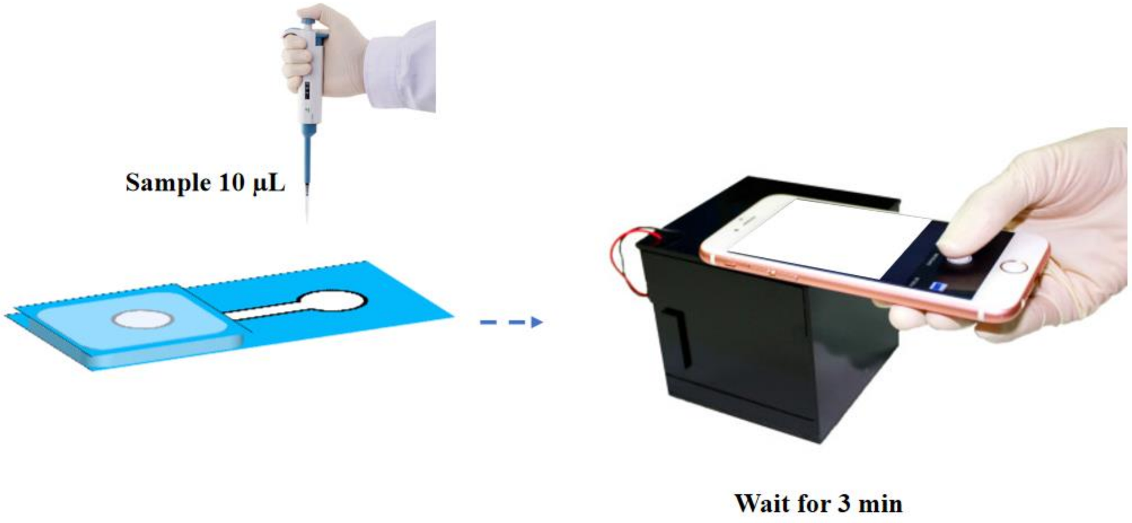

Figure 2.6 The detection procedure using the proposed device (pattern I)

For the pattern II, 2 µL Au-AA solution was added in Au-AA inlet zone after applying 10 µL As(III) sample solution in the sample inlet zone. After 3 min, the color change image was also captured using a smartphone and analyzed the color intensity by Image J software.

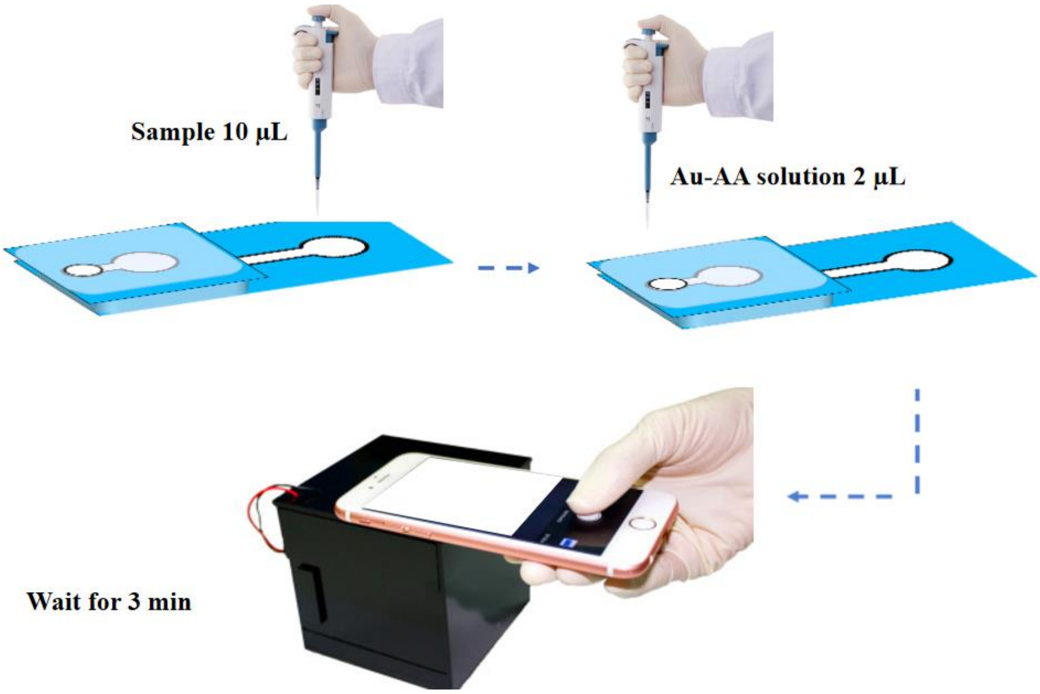

Figure 2.7 The detection procedure using the proposed device (pattern II)

Chapter 3

Results and discussion

3.1 Detection principle

3.1.1 Colorimetric paper-based device for As(III) detection using Ag(I) as a colorimetric reagent

Firstly, the pattern I was used to study the colorimetric reaction properties utilizing 1,000 ppm Ag(I) as the recognizing element in the detection zone for determination of As(III). As shown in Figure 3.1, it can be clearly seen that there is no color change in blank solution (MilliQ water) as a tester. Meanwhile, when As(III) solution was introduced in the sample inlet zone, it could change the color of Ag(I) from colorless to yellow in the detection zone. The colorimetric reaction mechanism relies on the BH_4^- ions from 5 %w/v $NaBH_4$, which could reduce As(III) to AsH_3 gas (arsine gas) in an acidic solution (5 M HCl). The arsine gas is then reduced Ag(I) to Ag(0) in the detection zone and caused the distinctive color change of the Ag(I) from the colorless to yellow (Ag(0)). This change can be visualized by the naked eye within 10 min following below equations[5]:

$$H_3AsO_3 + 2BH_4^- + 2H^+ \rightarrow 2AsH_3 + 2H_3BO_3 + 3H_2 \quad (1)$$

$$AsH_3 + 6AgNO_3 \rightarrow AsAg_3{\cdot}3AgNO_3 + 3HNO_3 \quad (2)$$

$$AsAg_3{\cdot}3AgNO_3 + 3H_2O \rightarrow H_3AsO_3 + 6Ag^0 + 3HNO_3 \quad (3)$$

Figure 3.1 The visual image of the detection zone for the determination of As(III) at the concentration of 0, 500 and 1,000 ppm using 1,000 ppm Ag(I) as a color reagent with a reaction time of 10 min.

However, the color intensities of As(III) at concentrations of 500 ppm and 1000 ppm were not significantly different. Furthermore, Ag(I) itself is easily oxidized in the air at room temperature. The more stable recognizing element such as Au(III) was, therefore, taken into consideration.

3.1.2 Colorimetric paper-based device for As(III) detection using Au(III) as a colorimetric reagent

Au(III) offers a similar property to Ag(I) in the colorimetric reactions with the arsine gas. However, Au(III) is more stable than Ag(I) in the air. In an acidic solution, As(III) could react with $NaBH_4$ and produce arsine gas. Following the same protocols, 5 M HCl and 5%w/v $NaBH_4$ were applied in PADs whereas 1,000 ppm Ag(I) was replaced with Au(III) on the detection zone instead. The violet products could be observed in the detection zone when the generated arsine gas reduces Au(III) to Au(0), as shown in Figure 3.2. The color darkened gradually from colorless to violet, corresponding to the As concentrations (100 ppm, 500 ppm and 1000 ppm). However, the sensitivity of this proposed device is still not good for the As(III) determination in a real sample. Thus, an additional signal enhancement method was required to enhance the sensitivity of As(III) detection.

Figure 3.2 The visual image of the detection zone for the determination of As(III) at the concentration of 0, 100, 500 and 1,000 ppm using 1,000 ppm Au(III) as a color reagent with a reaction time of 10 min.

3.1.3 Colorimetric paper-based device for As(III) detection using Au(III) as a color reagent and Au-enhanced Au particles

To improve the sensitivity of the sensor, the Au-enhanced Au(0) particles method was applied in this proposed device (Pattern II) using the mixture of 5 ppm Au(III) and 5 mM AA (Au-AA) solution (given as ratio 1). Au(III) could be reduced to Au(0) by AA solution and then adhered to the surface of Au(0). Hence, the size of Au(0) particles will be increased. Moreover, the enlarged surface area of Au(0) could improve the color change signal. To confirm the performance of the Au-enhanced Au particles method, 2 µL of MilliQ water and Au-AA solution were added to the Au-AA inlet zone of each device as a blank solution and the obtained results were then compared. The differences between the mean color intensity values after the addition of blank solution and As(III) standard solution in the sample inlet zone ($\Delta I = I_{blank} - I_{sample}$) were calculated for determining As(III). As shown in Figure 3.3, the results show that the dark violet color of the Au(0) particle was observed on the Au-enhanced Au particles, meanwhile that of the blank solution provided much lower ΔI than the Au-AA solution. Therefore, the Au-enhanced Au particles method was chosen in this proposed device for the determination of As(III).

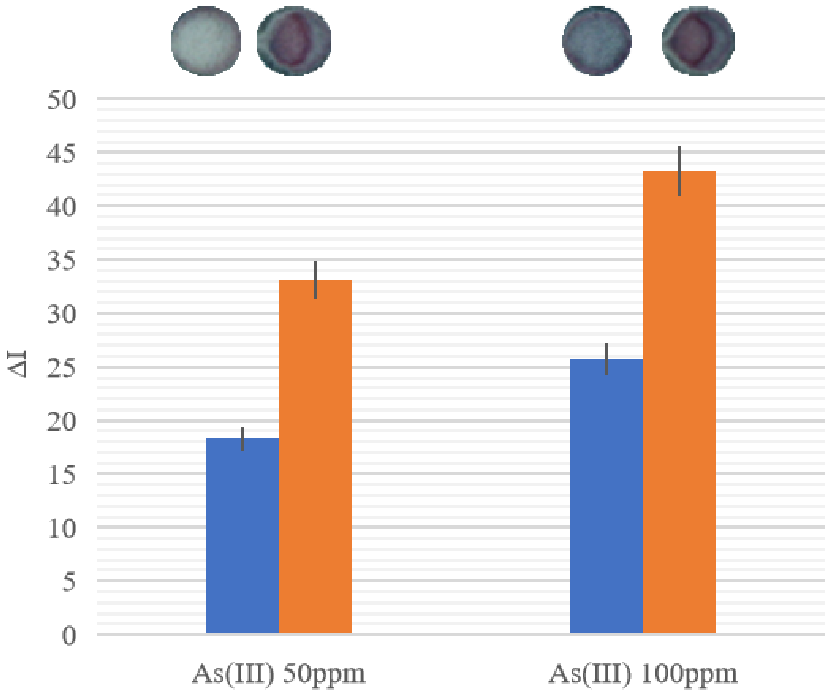

Figure 3.3 The visual image of the detection zone and the bar graph of ΔI for the determination of As(III) at the concentration of 50 and 100 ppm using 1,000 ppm Au(III) as a color

reagent after adding MilliQ water (blue bar) and Au-AA solution (orange bar) with a reaction time of 10 min.

3.2 Optimization of the assay conditions

The performance of the colorimetric paper-based sensor is influenced by the various assay conditions, including the concentration of Au(III) in Au-AA solution, the concentration of Au(III) as a color reagent, the concentration of HCl, the concentration of $NaBH_{4,}$ and the detection time. These parameters were optimized using 50 ppm of As(III) in this work in order to obtain the maximum detection efficiency of the sensor.

3.2.1 The effect of the concentration of Au(III) in Au-AA solution for the Au-enhanced Au particles method

The Au-enhanced Au particles method was performed in the developed device to improve the sensitivity of the sensor. Therefore, the performance of the developed device is influenced by the concentration of Au(III) in Au-AA solution (volume ratio of Au(III) and 5 mM AA = 1). For the effect of Au(III) concentration, different levels in 0.5, 1 and 5 ppm were investigated, as shown in Figure 3.4. The highest intensity was obtained in a 1 ppm Au(III) containing 5 mM AA solution. The higher concentration of Au(III) solution could provide a high intensity of violet color. Likewise, the intensity of the blank was also proportionally high as well. We believed that the excess amount of Au(III) could also be reduced to Au(0) particles in the detection zone by AA before arsine gas comes reacted with Au(III). Therefore, 1 ppm Au(III) and 5 mM AA solution given as volume ratio 1:1 was chosen for Au-enhanced Au particles in this work.

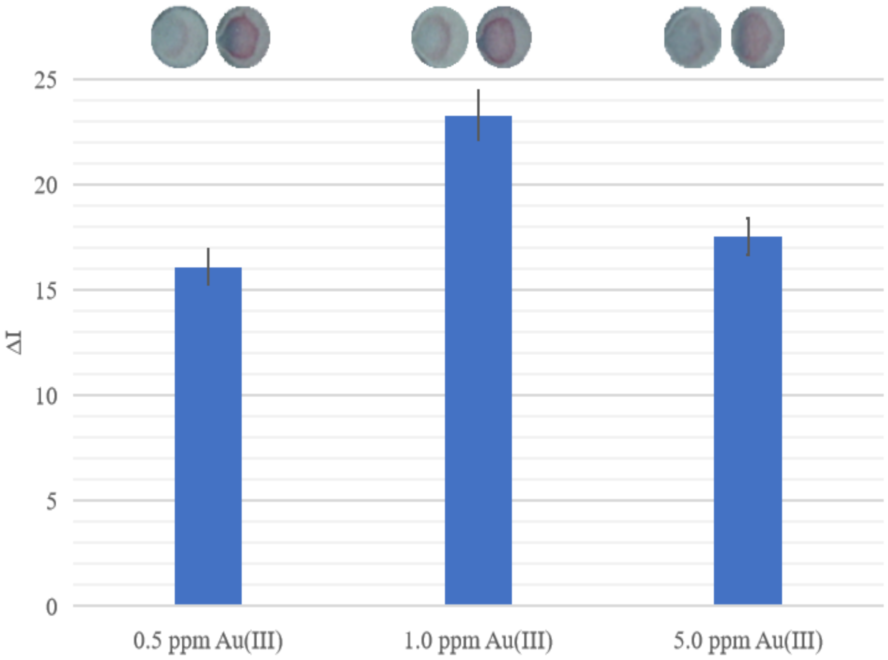

Figure 3.4 The effect of Au(III) concentration (0.5, 1 and 5 ppm) with fixed AA concentration (5 mM) on the analytical performance of PADs. (conditions: 10 µL of 50 ppm As(III) with a measurement time of 10 min)

3.2.2 The effect of the concentration of HCl

Since arsine gas from hydride generation reaction must be performed in acidic conditions, therefore, the effect of HCl concentration was studied in this work by varying the concentration in the range of 2.5 - 7.5 M, as shown in Figure 3.5. From the results, it was observed that there was a gradual decrease in ΔI when increasing the concentration of HCl. The highest intensity was observed in 2.5 M HCl, leading to the highest sensitivity. No significant difference intensity between 2.5, 5.0 and 7.5 M HCl for As(III) 50 ppm testing was observed. Furthermore, at low HCl concentration, the detection zone tended to have not change of color over time after adding a blank solution in the sample inlet zone. In this system, the excess HCl will react with $NaBH_4$ and produce H_2 gas. This produced H_2 gas will oxidize Au(III) to Au(0) as the redox potential of H^+/H_2 is lower than that of Au(III)/Au(0), leading to the presence of violet color in the detection zone after adding blank solution. Therefore, a HCl concentration of 2.5 M was applied in the developed device.

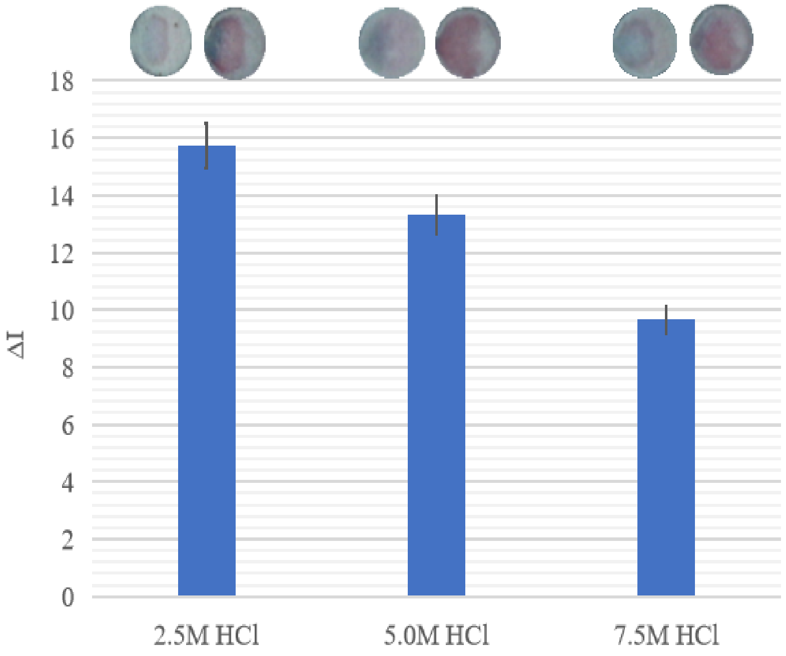

Figure 3.5 The effect of the concentration of HCl (2.5, 5 and 7.5 M) on the analytical performance of PADs. (conditions: 10 µL of 50 ppm As(III) with a measurement time of 10 min)

3.2.3 The effect of the concentration of Au(III)

The Au(III) was used as the recognition element to take part in the colorimetric reaction with arsine gas, whereas the Au(III) from the Au-enhanced Au particles was used to improve the sensitivity. Given that the Au(III) could act as the colorimetric reagent for As(III), the concentration of Au(III) was studied. The responses obtained from the different Au(III) concentrations were illustrated in Figure 3.6. The results of color change obtained by 1,500 ppm Au(III) provided the highest sensitivity. At the higher Au(III) concentration, the amount of As(III) reacting with Au(III) is limited, thus lowering the sensitivity. Therefore, the optimal concentration of Au(III) of 1,500 ppm was used in this work.

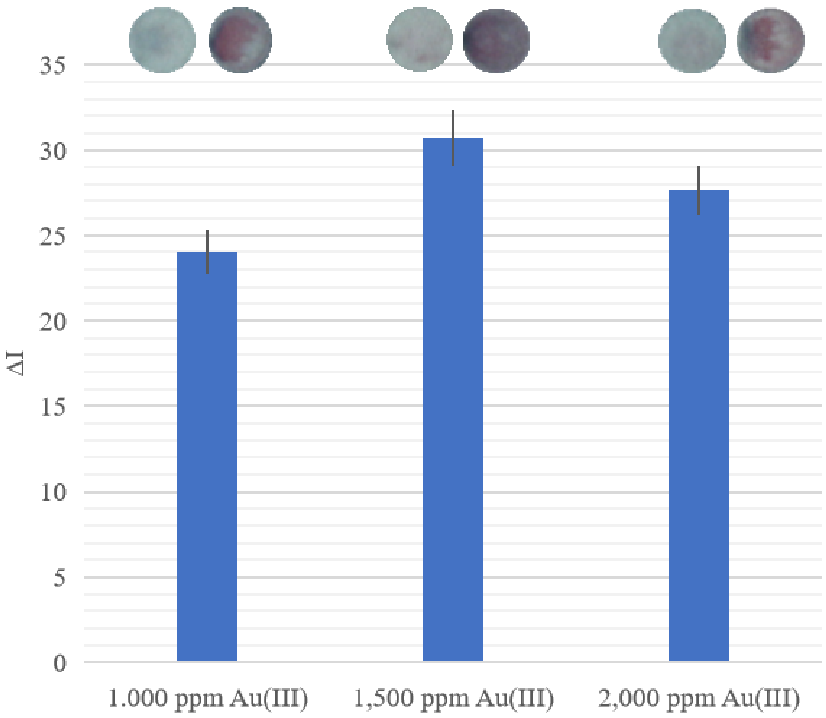

Figure 3.6 The effect of Au(III) concentration (1,000, 1,500 and 2,000 ppm) on the analytical performance of PADs. (conditions: 10 µL of 50 ppm As(III) with a measurement time of 10 min)

3.2.4 The effect of the concentration of $NaBH_4$

$NaBH_4$ is an important agent for arsine gas-produced by hydride generation reaction. It could react with As(III) to produce arsine gas. Hence, the effect of the concentration of $NaBH_4$ was investigated. The difference in the mean color intensity values (ΔI) responded to the different concentration of $NaBH_4$ is shown in Figure 3.7. At a high concentration of $NaBH_4$, the violet color of the detection zone became darker which led to a high color intensity value as a number of BH_4^- ions can entirely react with the excessive amount of As(III), leading to high sensitivity. Hence, the $NaBH_4$ concentration was chosen at 7.5% for the following experiment.

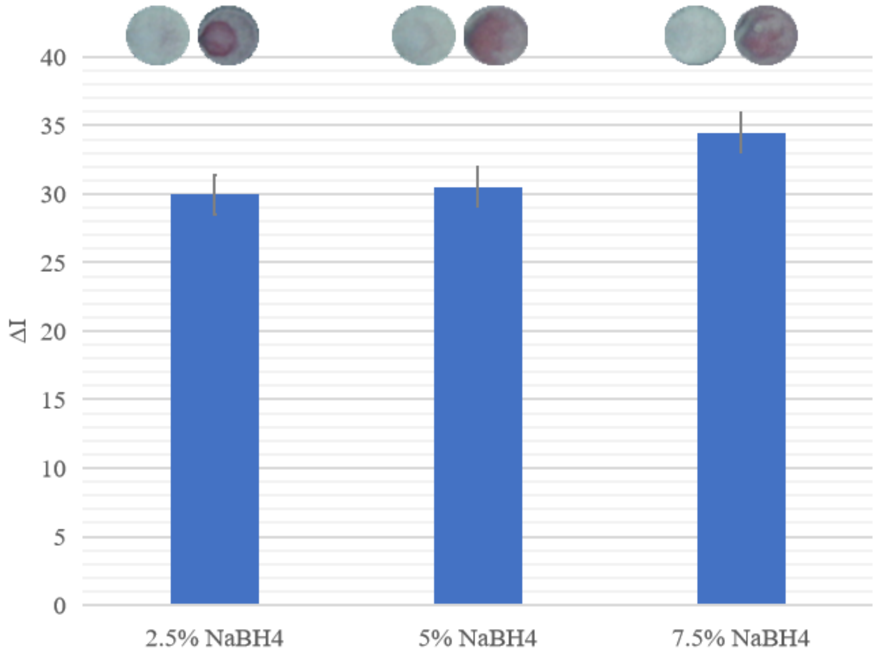

Figure 3.7 The effect of the $NaBH_4$ concentration (2.5, 5 and 7.5%) on the analytical performance of PADs. (conditions: 10 µL of 50 ppm As(III) with a measurement time of 10 min)

3.2.5 The effect of the measurement time

The detection time also plays an essential role in the colorimetric results. The relationship between intensity changed and measurement time up to 20 minutes were studied, as illustrated in figure 3.8. According to the bar graphs, the mean color intensity values on the detection zone after the addition of As(III) 50ppm samples increased to 3 min. Beyond 3 min, the difference intensity slightly declined. Thus, the measurement time of 3 min was chosen for the following experiment.

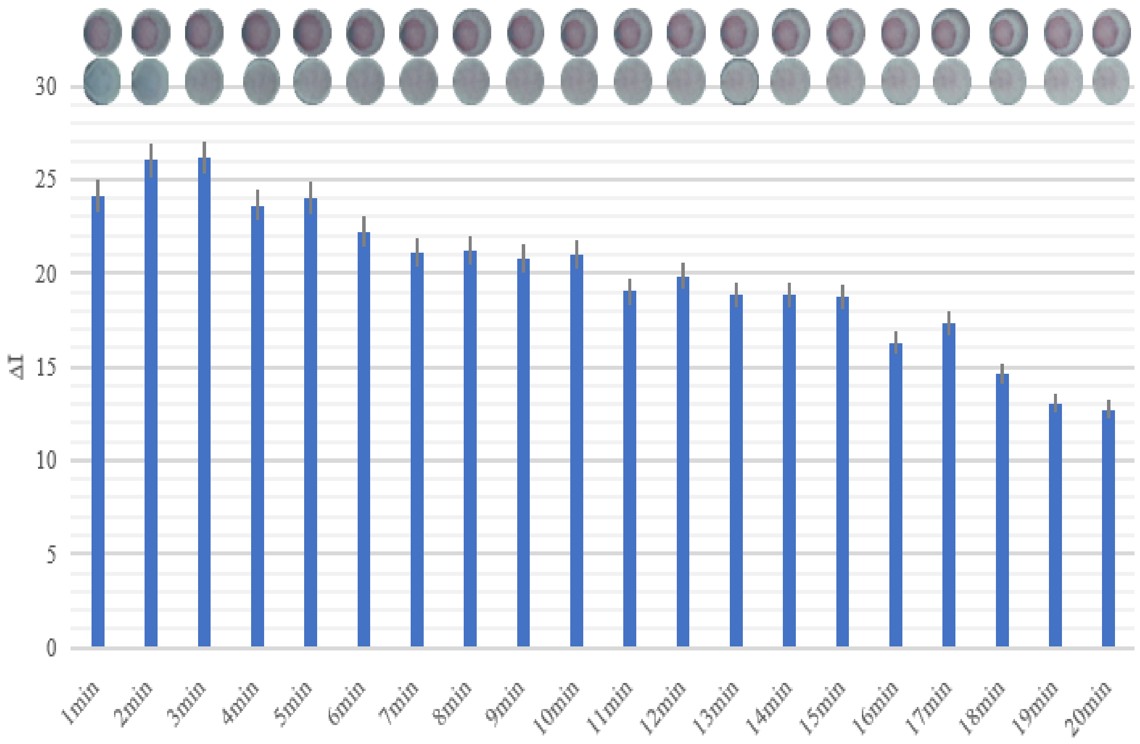

Figure 3.8 A Plot between mean color intensity and measurement time ranging up to 20 min in the presence of As(III) 50 ppm.

3.3 Analytical performance

In order to measure As(III) on a paper-based analytical device with the Au-enhanced Au particles, the performance of the proposed method was investigated. With the addition of the As(III), the mean color intensity of the Au(III) with the Au-Enhanced gold increased gradually, corresponding to the color changed from white to dark violet (as shown in Figure 3.9). Under optimal conditions, the mean color intensity measured from ImageJ program is related linearly to the logarithmic concentration of the total As samples (log As(III) concentration) in the range of 5 to 1000 ppm) (R^2= 0.993) as shown in Figure 3.10 with a limit of detection (LOD) and limit of quantitation (LOQ) from a calculation of 1.6 ppm and 5.4 ppm, respectively. This indicates that the proposed sensor can be used for the quantitative analysis of As(III).

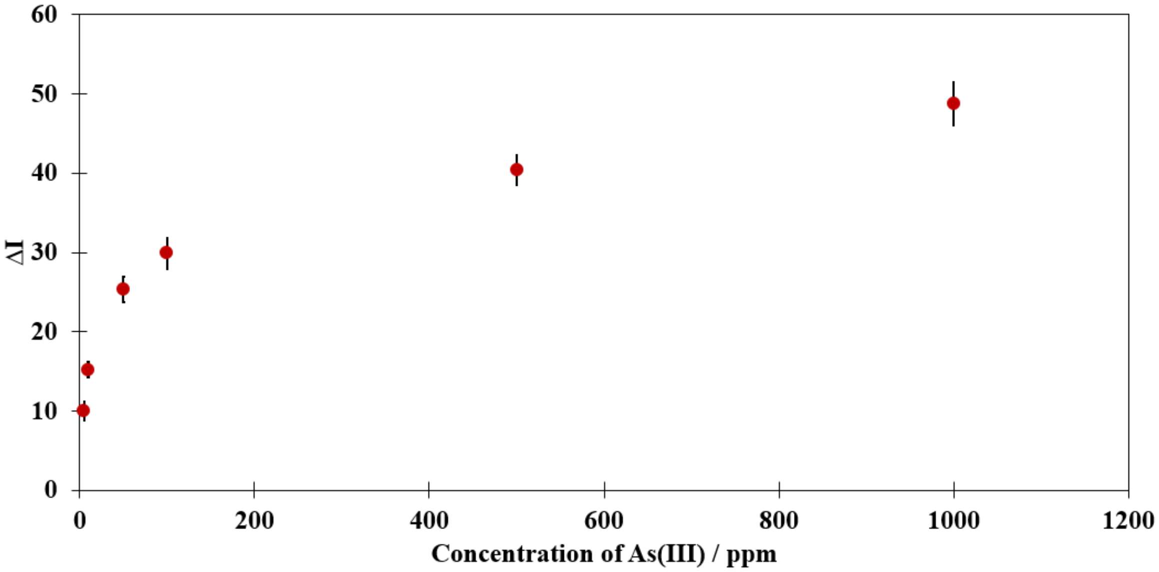

Figure 3.9 The plot of the mean color intensity of the Au(III) with Au-enhanced Au particles determined by digital image analysis using ImageJ program versus As(III) concentration (5-1000 ppm).

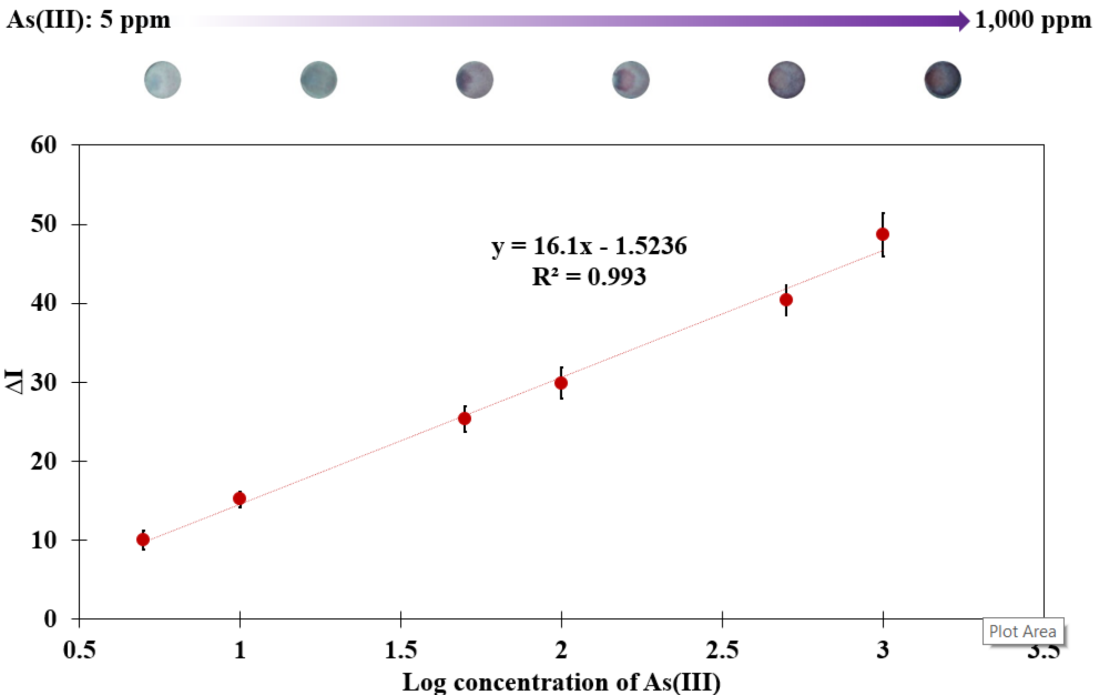

Figure 3.10 The linear plots between the mean color intensity and logarithmic As(III) concentration (optimal condition: Au(III) concentration in Au-AA solution of 1 ppm, Au(III) concentration as a color reagent of 1,500 ppm, HCl concentration of 2.5 M, $NaBH_4$ concentration of 7.5% and measurement time of 3 min).

Table 3.1 Comparison between analytical methods for As(III) determination

Nano-material	Detection method	Analyte	Limit of detection (LOD) (ppm)	Linearity (ppm)	Ref
CTAB with AuNPs	Colorimetric method	As(III)	0.0169	0.001-0.1	[14]
Functionalized AuNPs	Colorimetric method	As(V)	0.0025	0.02-0.2	[15]
AuNPs	Colorimetric method	As(III)/ As(V)	0.002	0.005-0.5	[16]
Exonuclease	Colorimetric method	As(III)	0.00002	0.0001-0.2	[17]
AuNPs	Colorimetric method	As(III)	1.6	5-1,000	Present work

The analytical performance was compared to those previous reports (Table 3.1). Those techniques provided lower LOD or high sensitivity. However, the wider linearity of this work means the proposed device is available to screen As(III) content in the real samples for on-site analysis. Moreover, the proposed PAD exhibits the advantages of disposability, portability, simplicity, and less amount of sample volume and reagent consumption.

3.4 Interferences study

To further assess the selectivity of the paper-based sensors, several environmental relevant heavy metals species including Cu(II), Cd(II), Zn(II), Pb(II), and Cr(IV) was first examined individually at 500 ppm in the presence of 50 ppm As(III). The results demonstrated that there was no significant difference between before and after adding interfering ions in As(III) 50 ppm solution. The results can be concluded that the hydride generation reaction between $NaBH_4$ and other species did not interfere with the following colorimetric reaction of As(III) and Au(III). Hence, it can be concluded that this proposed sensor can be applied to use in a real sample application with good selectivity.

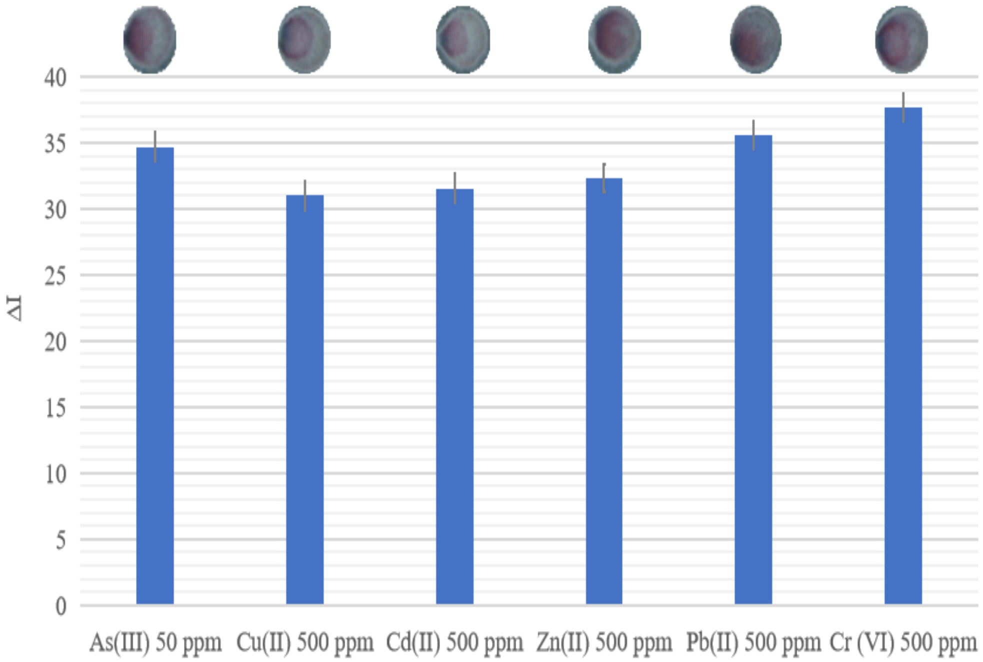

Figure3.11 the photographic image results of the colorimetric detection on PADs and the plot of the mean color intensity difference of the Au(0) on the detection zone after the addition of the mixture solution comprising As(III) 50 ppm and indicated interfering substances (500 ppm)

Chapter 4

Conclusion

By taking advantage of the unique optical properties of the Au(III), a new paper-based colorimetric approach for the determination of As(III) was successfully developed. The limitation of the sensitivity of PADs was addressed by the Au-enhanced Au particles. Moreover, the violet color of Au(0) particles (relate linearly with the concentration of As(III)) could be monitored by smartphone. All the operations for the determination of As(III) were performed in a single device, without the need for a sophisticated experimental procedure or instrument. Under optimal conditions, this colorimetric sensor exhibited good selectivity towards the As(III) against other heavy metal species. Considering the versatility and simplicity provided by the proposal, this colorimetric sensor is promising for on-site determining and screening of As(III) levels in an environmental area. In addition, this proposed device can be expected that similar devices for other analytes will be developed in the near future where these PADs may serve as a template.

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Biography

Ms. Yinxi Zhu was born on 27 June 1998 in Taiyuan, Shanxi, China. In 2017, she graduated from the Shanxi Experimental Secondary School of science division. Then, she continued to pursue her education in Thailand. After entering Chulalongkorn University, she chose applied chemistry as her major. Since year 2, she started the environmental study as the minor. She plans to focus on more specific study in environmental health and pursue a degree in public health in environmental health solution.

Yinxi Zhu