

**DEVELOPMENT OF DIGITAL COLORIMETRIC  
DETECTION TECHNIQUE FOR ARSENIC (III),  
ARSENIC (V) AND TOTAL INORGANIC  
ARSENIC**

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**DEVELOPMENT OF DIGITAL COLORIMETRIC DETECTION TECHNIQUE  
FOR ARSENIC (III), ARSENIC (V) AND TOTAL INORGANIC ARSENIC**

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## ABSTRACT

Arsenic is one of heavy metals that are associated with the main threats to human health. Therefore, determination of inorganic arsenic on sites is very important. Although arsenic field kits based on Gutzeit method have been extensively used for arsenic determination in the fields, however the major drawback of this approach is poor reproducibility causing a considerable determination error as the measurement of the coloured complex formed is done by the naked eye. Thus, enhancement of the accuracy and precision of the arsenic detection method is required.

In this study, a simple and rapid colorimetric quantitative method was developed based on a colour complex formed by silver ion impregnated on a filter paper with arsine gas produced from arsenic ion reduction in the sample by hydrogen generated from zinc and sulfamic acid reaction. The images of the color compounds were captured and digitised into Red (R), Blue (B), and Green (G) colour values. Experiments with incorporation of image processing technique into the colorimetric method were conducted to determine the significant factors and optimum conditions for detection of arsenic (III), arsenic (V) and total inorganic arsenic in water samples. Mathematical models were developed for estimation of arsenic (III), arsenic (V) and total inorganic arsenic. Besides that, determination of arsenic (III), arsenic (V) and total inorganic arsenic in the real water samples and spiked tap water samples were also performed using laboratory instrument and the newly developed method. The significant factors influenced the detection of arsenic (III),

arsenic (V) and total inorganic arsenic were found to be mass ratio of sulfamic acid to zinc powder followed by reaction period and mass ratio of sulfamic acid to zinc powder-reaction period-drying period of silver nitrate-impregnated filter paper interaction. The optimum conditions to determine arsenic (III), arsenic (V) and total inorganic arsenic were: 1.00 g of sulfamic acid, 0.50 g zinc powder and 5.0 minutes of reaction period; 3.00 g of sulfamic acid, 1.50 g of zinc powder and 5.0 minutes of detection period; and 1.50 g for sulfamic acid, 0.75 g zinc powder and 5.0 minutes of detection period.

From the interference study, it was found that the presence of various ions did not give significant effect on the arsenic determination. The method detection limits of arsenic (III), arsenic (V) and total inorganic arsenic were relative low i.e. 2 µg/L, 3 µg/L and 2 µg/L respectively. The accuracies and precisions of the methods for arsenic (III), arsenic (V) and total inorganic arsenic determinations in water samples were high as the relative standard deviations (RSD)s and the relative biases (RB)s for detection of arsenic (III), arsenic (V) and total inorganic arsenic in ground water and lake water samples were found in the ranges of 3%-15% and 4%-12%, respectively.. The recoveries of arsenic (III), arsenic (V) and total inorganic arsenic in spiked tap water samples were high (90.30% to 104.40%) and comparable with that of (97.65 to 99.50%) HPLC-ICP-MS.

## ABSTRAK

Arsenik merupakan salah satu logam berat yang sangat berbahaya kepada kesihatan manusia. Maka kaedah untuk penentuan kepekatan arsenik tak organik di lapangan adalah penting. Walaupun kit ujian arsenik berdasarkan kaedah Gutzeit telah digunakan secara meluas untuk penentuan kepekatan arsenic tak organik di lapangan, namun kelemahan utama pendekatan ini adalah ia mempunyai ralat yang besar yang disebabkan oleh penentuan arsenik berdasarkan kompleks berwarna yang terbentuk dengan menggunakan mata kasar. Justeru itu, penambahbaikan dari segi ketepatan dan kejituan kaedah penentuan arsenik adalah dititikberatkan.

Dalam kajian ini, satu kaedah kuantitatif kolorimetri yang ringkas dan cepat berdasarkan warna kompleks yang terbentuk daripada tindakbalas antara ion perak yang terjerap pada kertas turas dengan gas arsin yang terhasil daripada proses penurunan ion arsenik dalam sampel oleh hidrogen yang dihasilkan daripada tindakbalas antara zink dan sulfamik asid. Imej kompleks yang terbentuk didigitalkan kepada nilai digital merah (R), biru (B), dan hijau (G). Eksperimen dengan penggabungan teknik pemprosesan imej ke dalam kaedah kolorimetri telah dijalankan untuk menentukan faktor-faktor yang signifikan dan keadaan optimum untuk menentukan kepekatan arsenik (III), arsenik (V) dan total tak organik arsenik dalam sampel air. Model matematik telah dibangunkan untuk menganggar kepekatan arsenik (III), arsenik (V) dan total tak organik arsenik. Pelbagai ion telah digunakan dalam kajian gangguan untuk mengkaji kesan ion-ion tersebut ke atas penentuan

kepekatan arsenik (III), arsenik (V) dan total tak organik arsenik. Selain itu, penentuan arsenik (III), arsenik (V) dan total tak organik arsenik dalam sampel air sebenar dan sampel air paip yang ditambahkan arsenik juga dilakukan dengan menggunakan instrumen makmal dan teknik yang dibangunkan.

Faktor yang signifikan dalam penentuan kepekatan arsenik (III), arsenik (V) dan total tak organik arsenik adalah nisbah jisim asid sulfamik kepada serbuk zink, diikuti dengan tempoh tindakbalas dan kesan interaksi nisbah jisim asid sulfamik kepada serbuk zink, dan tempoh tindakbalas-tempoh pengeringan kertas turas terjerap perak nitrat. Manakala keadaan optimum untuk menentukan kepekatan arsenik (III), arsenik (V) dan total arsenik adalah: 1.00 g asid sulfamik, 0.50 g serbuk zink dan 5.0 minit tempoh tindakbalas; 3.00 g asid sulfamik, 1.50 g serbuk zink dan 5.0 minit tempoh tindakbalas serta 1.50 g asid sulfamik, 0.75 g serbuk zink, dan 5.0 minit tempoh tindakbalas.

Daripada kajian gangguan dengan menggunakan pelbagai ion-ion, didapati bahawa tiada kesan signifikan ke atas penentuan kepekatan arsenik. Had pengesanan kaedah arsenik (III), arsenik (V) dan total tak organik arsenik adalah rendah secara relatif iaitu masing-masing 2 µg/L, 3 µg/L dan 2 µg/L. Ketepatan dan kejituan kaedah yang dibangunkan untuk menentukan kepekatan arsenik (III), arsenik (V) dan total tak organik arsenik di dalam sampel air adalah tinggi disebabkan sisihan piawai relatif (RSD) dan berat sebelah relatif (RB) untuk mengesan kepekatan arsenik (III), arsenik (V) dan

total tak organik arsenik dalam sampel air bawah tanah dan sampel air tasik masing-masing adalah di antara 3% -15% dan 4% -12%. Perolehan semula arsenik (III), arsenik (V) dan total tak organik arsenik dalam sampel air paip yang ditambah arsenik adalah tinggi (90.30% kepada 104.40%) dan setanding dengan kepekatan arsenik yang ditentukan dengan (97.65% kepada 99.50%) alat HPLC-ICP-MS.

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## APPROVAL

I certify that an Examination Committee has met on **8 MARCH 2017** to conduct the final examination of **LEONG JIN HOONG** on his degree thesis entitled "**DEVELOPMENT OF DIGITAL COLORIMETRIC DETECTION TECHNIQUE FOR ARSENIC (III), ARSENIC (V) AND TOTAL INORGANIC ARSENIC**". The committee recommends that the student be awarded the **Doctor of Philosophy (Chemical Engineering)**.

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**LIST OF ABBREVIATIONS**

<b>ANOVA</b>	Analysis of Variance
<b>B</b>	Blue
<b>DOE</b>	Design of Experiment
<b>DP</b>	Drying period of silver nitrate-impregnated filter paper
<b>DW</b>	Drying Weight load used for drying silver nitrate-impregnated filter paper
<b>ETV</b>	US Environmental Protection Agency's Environmental Technology Verification
<b>FDA</b>	U.S. Food and Drug Administration
<b>G</b>	Green
<b>HPLC-ICP-MS</b>	High Performance Liquid Chromatography coupled
<b>ICP-MS</b>	Inductively coupled plasma mass spectrometry
<b>LoD</b>	Limit of detection
<b>LOD/MDL</b>	Theoretical limit of detection/method of detection limit
<b>MDL</b>	Method detection limit
<b>MSZ</b>	Mass ratio sulfamic acid to zinc powder
<b>R</b>	Red
<b>RB</b>	Relative Bias
<b>RGB</b>	Red Green Blue
<b>RP</b>	Reaction Period
<b>RSD</b>	Relative Standard Deviation
<b>USEPA</b>	United States Environmental Protection Agency
<b>WHO</b>	World Health Organisation
<b>ICP-MS</b>	Inductively Coupled Plasma Mass Spectrometer
<b>XRF</b>	X-ray fluorescence

## CHAPTER 1

### INTRODUCTION

Arsenic is one of heavy metals that are associated with the main threats to human health (Jarup, 2003). Inorganic arsenic contamination has caused a remarkable impact on the contamination of soil and groundwater in many countries of the world (Kathleen *et al.*, 2011) and it is reported that more than 140 million people worldwide are exposed to excessive arsenic, mainly by drinking ground waters contaminated by inorganic arsenic which is more toxic than organic arsenic (Fendorf *et al.*, 2010; Ravenscroft *et al.*, 2009). Chronic consumption of arsenic contaminated water causes skin lesions, neurological disorders and cancers, including cancer of the kidneys and lungs (Sambu and Wilson, 2008).

Arsenic occurs in natural water in various inorganic and organic forms (Cullen and Reimer, 1989) and mainly found in two inorganic forms i.e.  $\text{As}^{3+}$  and  $\text{As}^{5+}$ , whereby arsenic (III) is more toxic than As (V) (Mizumura *et al.*, 2010). According to Smedley and Kinniburgh (2013), inorganic arsenic (III) and arsenic (V) are commonly found in surface water, groundwater, rainwater, rocks, unconsolidated sediments and soils. Its toxicity changes mainly on the chemical form in which it is present as caused by the variations in the solubility, physical state, rate of absorption and elimination of the species (Nath *et al.*,

2014). Therefore, it is crucial to determine each inorganic arsenic species in the water samples.

Laboratory instruments such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can be used to quantify trace arsenic (Sengupta and Dasgupta, 2009) but these instruments are bulky, expensive and too sophisticated to be used in the fields. Thus, it is desirable to develop a method for the determination of inorganic arsenics on site. Consequently, various arsenic test kits have been developed based on colorimetric method by modifying the Gutzeit method for arsenic determination in groundwater. However, most of the test kits are unreliable for detection of arsenic at the concentration close to the arsenic toxicity concentration threshold (Yogarajah and Tsai, 2015). The major drawback of these approaches are poor reproducibility which causes a considerable determination error of 20% to 35% due to measurement of the coloured complex formed using the naked eye (Abrazhee *et al.*, 2002) which is rather subjective and operator-dependent.

According to Wang *et al.* (2011), an ideal field deployable sensor would be a technique that able to detect low  $\mu\text{g/L}$  concentration of analyte directly on-site with little or no sample preparation as well as persistence to interference ions. In fact, colorimetric methods can provide results nearly as accurate and reliable as those from analytical laboratories when the reaction is automatically evaluated by means of a color detector (Safarzadeh-Amiri *et al.*, 2011). An electronic device for measuring colour has been introduced to minimise human error in interpreting the colour with naked eyes for the



determination of arsenic. For example, Anderson *et al.* (2008) measured reflectance of the developed colour spot and converted it to a digital signal using an electronic transducer which requires 30 to 40 minutes to detect arsenic.

Besides that, Carro Perez and Francisca (2013) reported that the accuracy of arsenic detection using (Macherey–Nagel Quantofix) Arsen10 ® and Arsen 50 ® arsenic test kits increased significantly by digitalising the colour images formed on the paper strips using an image processing technique. The arsenic test kits were able to detect arsenic concentrations as low as 0.065 mg/L, and, for 10 µg/L, the relative error is  $\pm 6.2$  µg/L with a 95% confidence level. However, the chemicals used are concentrated hydrochloric acid (HCl) and mercuric (II) bromide, which are difficult to handle during transportation and when disposed concentrated HCl is highly corrosive and mercuric (II) bromide is a toxic compound.

Most of the digital colorimetric detection techniques detection is limited for arsenic (III) determination in water samples as tabulated in Table 1. In addition, chemical used for reaction comprises of those toxic chemicals and highly corrosive such as hydrochloric acid and mercuric (II) bromide with a prolong detection period from 10 minutes up to 30 minutes to determine the final result.