

## Digital Image-Based Colorimetry for Enhancement of Arsenic(III) Determination

JIN HOONG LEONG<sup>1</sup>, KEAT KHIM ONG<sup>2,\*</sup>, WAN YUNUS WAN MD ZIN<sup>3</sup>, FITRIANTO ANWAR<sup>4</sup>,  
CHIN CHUANG TEOH<sup>5</sup>, JAHWARHAR IZUAN ABD RASHID<sup>2</sup> and MANSOR AHMAD<sup>4</sup>

<sup>1</sup>Faculty of Engineering, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000 Kuala Lumpur, Malaysia

<sup>2</sup>Centre for Defence Foundation Studies, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000 Kuala Lumpur, Malaysia

<sup>3</sup>Faculty of Defence Science Technology, Universiti Pertahanan Nasional Malaysia, Kem Sungai Besi, 57000 Kuala Lumpur, Malaysia

<sup>4</sup>Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

<sup>5</sup>Malaysian Agricultural Research and Development Institute Headquarter, G.P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

\*Corresponding author: E-mail: ongkhim@upnm.edu.my

Received: 17 November 2017;

Accepted: 27 February 2018;

Published online: 31 May 2018;

AJC-18912

In this report, we describe a simple, rapid and reliable on-site detection method of arsenic(III) using colorimetric method incorporated with image processing technique. The method detection limit (2.24 µg/L), accuracy (relative bias of 0.5 to 5.2 %), precision (relative standard deviations of 0.2 to 3.3 %) of the developed mathematical model to estimate concentration of arsenic(III) were reported in this study. Interference by various ions were investigated and insignificant interferences were found.

**Keywords:** Arsenic(III), Colorimetric, Image processing.

### INTRODUCTION

Arsenic and its derivatives have been used in pharmaceutical products, pesticides, alloying agents, semiconductors and metal adhesives [1]. However, inorganic arsenic compounds are categorized in Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [2]. Arsenic can enter our body either by drinking of contaminated water or by the consumption of plants commonly grown in the contaminated area [3]. Environmental exposure to arsenic causes a huge health problem globally [4].

Various arsenic detection techniques were developed [5]. However, most of these methods have limitations such as interference by a large number of ions, low/moderate sensitivity and require heating or extraction from organic solvents, requirement of sample preservations and chemical reduction steps, which may introduce sample contamination [6]. In addition, the samples are required to be transported to a laboratory for the analysis using a sophisticated analytical instrument. These sophisticated analytical techniques need sample preparation before the analysis, which is found to be tedious, time consuming and more reagents are required [1,7].

Therefore, to overcome these disadvantages, a rapid and simple detection method is required to provide an on-site checking of risk level of source water for drinking use and of effluent from small-scale arsenic removal plants with a rapid

monitoring tool may be useful. Some kinds of spot test kits for detecting of arsenic using a test strip and a gas-detection tube were reported. These spot tests enable visual determination, but the accuracy is usually low as concentration of arsenic increases exponentially with the increase of colour intensity or colour band length [8]. Thus, there is a need to develop a simple, reliable, highly sensitive method, which would overcome the existing inadequacies in the determination of traces amounts of arsenic.

### EXPERIMENTAL

1000 mg/L of As(III) standard solution, silver nitrate, sulfamic acid and zinc powder were obtained from Merck (Germany) and used as received. All chemicals used were of analytical grade.

**Preparation of As(III) working standard solutions:** Various concentrations (0 to 300 µg/L) of As(III) working standard solutions were freshly prepared from the As(III) stock solution by dilutions using ultrapure water obtained from Milli-Q water purification system (Millipore).

**Preparation of 5 % (w/v) silver nitrate solution:** A 5 % (w/v) solution of silver nitrate was prepared by dissolving desired amount of silver nitrate in a 100 mL volumetric flask and made up with ultrapure water.

**Preparation of silver nitrate-impregnated filter paper:** A round-shaped piece of the paper with a diameter of 2.5 cm

was prepared by cutting Whatman filter paper No. 3. The paper was inserted into the silver nitrate solution for 2 seconds and then dried it by pressing it between two pieces of dry Whatman filter papers with a 100 load for 20 s. This silver nitrate-impregnated filter paper was used as the sensor paper for detection of As(III).

**Coloured compound formation:** The detection of various concentrations (0 to 300 µg/L) of As(III) was performed in five replications at the optimum conditions as reported by Leong *et al.* [9] in order to develop a mathematical model for the estimation of concentrations of As(III) in water samples. Briefly, the optimum amount of sulfamic acid was added to a 60 mL of polypropylene bottle which contained 50 mL of As(III) working standard solution. The bottle was swirled before adding the optimum amount of zinc powder and swirled again. The impregnated filter paper was subsequently inserted inside the cap of the bottle. The bottle was closed with the cap and swirled gently. The coloured impregnated filter paper was captured and used for image analysis after the optimum period.

**Colour image processing:** The image processing of the captured images were performed using the method as described by Leong *et al.* [9] where colour images were captured by a digital camera (Sony Cyber-shot, DSC-W610) at the distance of 15 cm at constant conditions for all experiments. The images were transformed into Red, Green and Blue (RGB) colour values (0 to 225) using Image J software and subsequently used for mathematical modelling and statistical analysis.

## RESULTS AND DISCUSSION

**Mathematical model development for As(III) determination based on RGB values:** A total of 30 experimental runs (n = 5 replicates) at six different concentrations of As(III) ranging from 0 to 300 µg/L were performed using the developed method. Using an image processing software, the images of coloured produced on the impregnated filter paper were analyzed and converted to obtain RGB values. The corresponding relative standard deviations (RSDs) and mean of each RGB values of each concentration are presented in Table-1. The RSDs of the RGB values at different concentrations of As(III) range from 1.03 to 4.81 % which less than 5 %, indicating that all data are precise and high reproducibility. Table-1 shows that all RGB values decrease as increasing of As(III) concentration. This indicated that the mean of RGB values are inversely proportional to the concentration of As(III).

TABLE-1  
MEAN OF RGB VALUES USED FOR  
MATHEMATICAL MODELLING

Known As(III) conc. (µg/L)	Mean of colour value			RSD (%)		
	R	G	B	R	G	B
0	140.84	161.01	164.02	1.03	1.04	1.27
10	138.90	152.10	158.90	2.00	2.17	3.09
50	134.53	147.10	148.04	3.03	2.65	4.81
100	129.90	132.50	123.56	3.32	3.06	4.21
200	120.59	125.76	94.62	3.78	3.92	3.58
300	105.88	106.99	79.12	3.48	3.69	4.41

R = Red, G = Green, B = Blue

Due to high precision of RGB data, the multiple regression analysis based on RGB values was employed in order to improve the colorimetric detection of arsenic in solutions. In this analysis, the mean of RGB values was used to develop a multiple regression model that can be used to determine the concentration of As(III) concentrations. The multiple regression model has the general form as presented in eqn. 1:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \varepsilon \quad (1)$$

where  $X_1$ ,  $X_2$ ,  $X_3$  denote the independent variables,  $Y$  stands for the dependent variable,  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  represent the correlation coefficients and  $\varepsilon$  designates the error term. With above equation, the final fitted model and the coefficient of RGB values were obtained and expressed in below equation:

$$\text{As(III) concentration} = 1009 - 6.70 \text{ Red} + 1.13 \text{ Green} - 1.511 \text{ Blue} \quad (2)$$

### Analytical performance of As(III) measurement

**Accuracy:** The accuracy of developed model for As(III) measurement was evaluated by relative bias (RB) as expressed by the following equation:

$$\text{RB (\%)} = \left| \frac{d}{C_R} \right| \times 100 \quad (3)$$

where  $d$  is the difference between the reading of As(III) concentration (µg/L) by the developed model and known As(III) concentration (µg/L) and  $C_R$  is known As(III) concentration. Overall, low RB was obtained as increasing of As(III) concentration from 10 to 300 µg/L as shown in Table-2. Table-2 shows that all the calculated As(III) concentration is almost too close to the references As(III) concentration. The results revealed that the RBs of calculated As(III) using the developed model were relatively low in the range from 0.5 to 5.2 %.

TABLE-2  
ACCURACY AND PRECISION OF DEVELOPED MODEL TO  
MEASURE As(III) WITH KNOWN As(III) CONCENTRATION

Known As(III) (µg/L)	Calculated mean As(III) (µg/L)	Relative standard deviation (%)	Relative bias (%)
10	10.52 ± 0.350	3.3	5.2
50	48.64 ± 2.160	4.4	2.7
100	103.40 ± 3.520	3.4	3.4
200	198.98 ± 2.367	1.2	0.5
300	301.420 ± 0.557	0.2	0.5

**Precision:** In addition, the determination of RSD values was employed to check the precision of the developed model in As(III) determination towards the references method (Table-2). By using 5 replicates of each As(III) concentration, the RSDs values were in the range of 0.2 % to 4.4 %. The results indicated that both relative bias and RSDs values were relative low. Due to smaller RB and RSD values obtained in this study, the measurement of As(III) by the developed model can be both accurate and precise.

**Linearity:** A further validation of developed model was examined by performing a linear regression of calculated As(III) against known As(III) concentration for the five samples ranging from 10 to 300 µg/L (Fig. 1) It was shown that the  $R^2$  of the linear regression for the developed model was 0.9998.

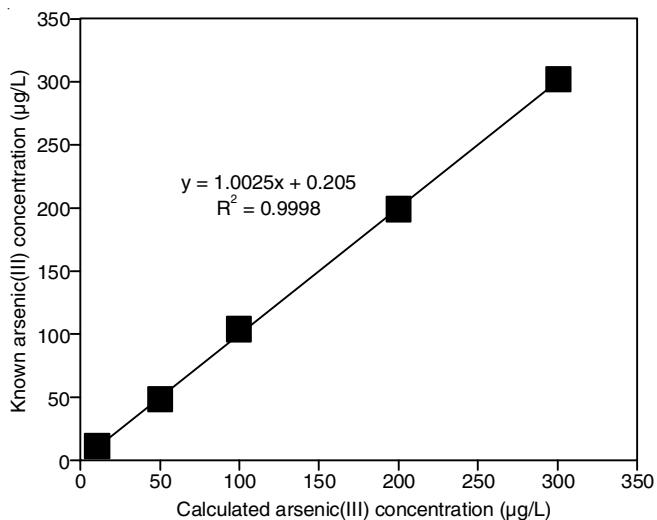


Fig. 1. Linear regression of calculated As(III) against known As(III) concentration

The high value of  $R^2$  revealed that the calculated As(III) concentration showed a good correlation with known As(III) concentration, thus validated the developed model to determine As(III) concentration. A similar technique was employed by Carro Perez and Francisca [10] and they concluded that the digital analysis image of paper strips for As(III) measurement is highly correlated ( $R^2 = 0.98$ ) with the standard method.

**Method detection limit (MDL):** The detection limit of As(III) measurement based on developed model was evaluated using method detection limit (MDL). The method detection limit is the minimum concentration of a substance measured and reported with 99 % confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte [11]. In this study, the minimum concentration of As(III) (10  $\mu\text{g/L}$ ) was employed to determine the method detection limit of As(III). The method detection limit for As(III) measurement based on developed model was performed by analyzing seven replicates of samples spiked with 10  $\mu\text{g/L}$  of As(III). The method detection limit was calculated based on the following equation:

$$\text{Method detection limit} = t \times \text{SD} \quad (4)$$

where  $t = 3.14$  is the Student's  $t$  value at 99 % confidence level with seven replicates and SD is the standard deviation of the replicate samples. The results of seven replications of the experiments to detect 9 and 10  $\mu\text{g/L}$  of arsenic(III) are presented in Table-3. The mean of 10  $\mu\text{g/L}$  of arsenic(III) was 10.097  $\mu\text{g/L}$  and recovery of 100.97 %. However, the mean of 9  $\mu\text{g/L}$  was only 4.502  $\mu\text{g/L}$  and recovery was also low (45.02 %). In addition, there was a significant difference between 9  $\mu\text{g/L}$  and the calculated arsenic(III) from the developed mathematical model. Consequently, the mean of 10  $\mu\text{g/L}$  was employed to calculate the method detection limit for detection of arsenic(III). The method detection limit was calculated by multiplying 3.14 with the standard deviation of 10  $\mu\text{g/L}$  of arsenic(III) based on eqn. 6. The method detection limit for detection of arsenic(III) was 2.24  $\mu\text{g/L}$ .

Hence, the developed model is able to determine As(III) concentration in the permissible limit of arsenic in drinking water by WHO. The detection limit of As(III) in this study

TABLE-3  
DETERMINATION OF ESTIMATED METHOD  
DETECTION LIMIT OF 10 AND 9  $\mu\text{g/L}$  OF As(III)

	Concentration of As(III)	
	9 $\mu\text{g/L}$	10 $\mu\text{g/L}$
Mean of calculated As(III) ( $\mu\text{g/L}$ )	4.502	10.097
Standard deviation ( $\mu\text{g/L}$ )	0.984	0.713
Mean of recovery (%)	45.02	100.97
$p$ -Value of paired sample $t$ -test	0.000	0.731
Method detection limit ( $\mu\text{g/L}$ )	–	2.24

was slightly lower (2.24  $\mu\text{g/L}$ ) as compared to the previous studies. Devi *et al.* [12] reported their arsenic field kit was able to detect arsenic as low as 4 ppb using Gutzeit method. Meanwhile, Hu *et al.* [13] employed the molybdenum method based on the formation of molybdenum blue complexes for detection of As(III)/(V) and found that the detection limit was 8  $\mu\text{g/L}$ . Using similar principle As detection in this study, Das and Sarkar [14] had achieved 10  $\mu\text{g/L}$  as the lowest detection limit of As(III) but without the use of image processing. Compared to our finding, the detection limit of As(III) measurement could be improved when coupling with image processing technique.

**Matrix interferences studies:** The efficiency of As(III) determination could be affected by the presence of ions in the real samples. Thus, the matrix interferences studies were performed by spiking synthetic samples contained 10  $\mu\text{g/L}$  of arsenic(III) with each ion individually at two different concentrations (*i.e.* low and high levels). The concentrations of these interferences ions used ranging from 0.002 to 400 mg/L (Table-4). The effect of these ions on the determination of As(III) could be evaluated based on the As(III) recoveries (%) as shown in Table-4. There is little or no loss of As(III) determination if the recovery value is close to 100 %. As it can be seen from Table-4, a good recovery of As(III) was observed which range from 93.84 to 115.07 % either using low or high-level concentration of the interference ions. These results suggested that either low or high-level concentration of the interference ions did not interfere in the determination of 10  $\mu\text{g/L}$  of As(III) using our developed model. This finding was supported with the Paired Sample  $t$ -test between known concentration of As(III) and calculated concentration of As(III) after spiked with interference ions at both levels. Due to the  $p$ -value of paired sample  $t$ -test for all interference metal ions at both levels was higher than 0.05 ( $p > 0.05$ ), there were no significant difference between known concentration of As(III) and calculated As(III). Interestingly, at a high-level concentration of  $\text{Na}^+$  (300 mg/L),  $\text{Cl}^-$  (200 mg/L),  $\text{SO}_4^{2-}$  (200 mg/L) and  $\text{NO}^-$  (400 mg/L) in the spiked samples are still exhibit a high recovery of As(III). Comparable with the study of Salman *et al.* [15] that claimed that the interferences metal ions such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$  gave insignificant effect on the determination of As in water. Similar observation was reported by Hagiwara *et al.* [16] and they concluded that the major and minor metal ions typically found in fresh water did not interfere with As recovery. This study also demonstrates that the determination of As(III) using this method could be applied to the water samples which contain the interference ions used in this study. Thus, it can be concluded that the combination of colorimetric

TABLE-4  
EFFECT OF INTERFERENCE IONS AT LOW AND HIGH LEVELS IN THE DETECTION OF As(III)

Interfering ion	Concentration (mg/L)	Calculated As(III) concentration Mean $\pm$ SD ( $\mu\text{g/L}$ )	Recovery (%)	<i>p</i> -value of Paired Sample <i>t</i> -test
Ni <sup>2+</sup>	0.002	10.303 $\pm$ 1.427	103.03	0.660
	0.200	11.037 $\pm$ 1.553	110.37	0.210
Mg <sup>2+</sup>	0.500	10.297 $\pm$ 1.496	102.97	0.680
	50.00	10.114 $\pm$ 1.132	101.14	0.399
Mn <sup>2+</sup>	0.005	11.293 $\pm$ 1.263	112.93	0.084
	0.500	10.33 $\pm$ 2.56	103.30	0.790
Al <sup>3+</sup>	0.003	11.330 $\pm$ 1.704	113.30	0.156
	0.300	11.507 $\pm$ 1.911	115.07	0.153
Fe <sup>3+</sup>	0.002	10.79 $\pm$ 1.808	107.90	0.384
	2.000	9.384 $\pm$ 1.389	93.84	0.378
Na <sup>+</sup>	3.000	10.26 $\pm$ 1.166	102.60	0.644
	300.0	10.713 $\pm$ 1.676	107.13	0.396
Cl <sup>-</sup>	2.000	10.381 $\pm$ 1.881	103.81	0.674
	200.0	10.911 $\pm$ 2.034	109.11	0.373
SO <sub>4</sub> <sup>2-</sup>	2.000	10.94 $\pm$ 2.38	109.40	0.428
	200.0	10.506 $\pm$ 1.375	105.06	0.457
NO <sub>2</sub> <sup>-</sup>	4.000	10.22 $\pm$ 1.290	102.20	0.859
	400.0	10.15 $\pm$ 1.032	101.50	0.892
NO <sub>3</sub> <sup>-</sup>	0.020	10.866 $\pm$ 1.492	108.66	0.264
	2.000	10.053 $\pm$ 1.723	100.53	0.948

method with the image processing technique can determine the level concentration of As(III) concentration accurately.

### Conclusion

Present method to determine arsenic(III) has been developed based on colorimetric method incorporated with image processing technique. Results indicated that the mean of RGB values were inversely proportional to the concentration of As(III). The final fitted model and the coefficient of RGB values for the presented method were obtained and tested with the accuracy (RBs of 0.5 to 5.2 %), precision (RSDs of 0.2 % to 4.4 %) and detection limit (2.24  $\mu\text{g/L}$ ). For the investigations on the interference with other ions, there is no significant interfere in the determination of 10  $\mu\text{g/L}$  of As(III) measured by the presented method of this study, using either low or high-level concentration of the interference ions.

### ACKNOWLEDGEMENTS

The authors express their gratitude to Ministry of Higher Education of Malaysia for the financial support (PRGS/1/2012/STWN01/UPNM/02/1). The authors also highly appreciated Universiti Pertahanan Nasional Malaysia for providing research facilities to conduct this study.

### REFERENCES

- K. Shrivastava, R. Shankar and K. Dewangan, *Sens. Actuators B Chem.*, **220**, 1376 (2015); <https://doi.org/10.1016/j.snb.2015.07.058>.
- R. Dominguez-Gonzalez, L. Gonzalez-Varela and P. Bermejo-Barrera, *Talanta*, **118**, 262 (2014); <https://doi.org/10.1016/j.talanta.2013.10.029>.
- L. Song, K. Mao, X. Zhou and J. Hu, *Talanta*, **146**, 285 (2016); <https://doi.org/10.1016/j.talanta.2015.08.052>.
- P. Mandal, *Emerging Contaminants*, **3**, 17 (2017); <https://doi.org/10.1016/j.emcon.2017.01.004>.
- J. Ma, M.K. Sengupta, D. Yuan and P.K. Dasgupta, *Anal. Chim. Acta*, **831**, 1 (2014); <https://doi.org/10.1016/j.aca.2014.04.029>.
- J. Saha, A.D. Roy, D. Dey, J. Nath, D. Bhattacharjee and S.A. Hussain, *Sens. Actuators B Chem.*, **241**, 1014 (2017); <https://doi.org/10.1016/j.snb.2016.10.098>.
- S.S. Boxi and S. Paria, *Dalton Trans.*, **44**, 20464 (2015); <https://doi.org/10.1039/C5DT03958D>.
- Y. Kiso, S. Asaoka, Y. Kamimoto, S. Tanimoto and K. Yokota, *J. Environ. Chem. Eng.*, **3**, 40 (2015); <https://doi.org/10.1016/j.jece.2014.11.017>.
- J. Leong, K. Ong, W. Zin, F. Anwar, U. Rauf, C. Teoh, H. Gapor and M. Osman, *Orient. J. Chem.*, **32**, 2407 (2016); <https://doi.org/10.13005/ojc/320512>.
- M.E. Carro Perez and F.M. Francisca, *J. Environ. Sci. Health A*, **48**, 191 (2013); <https://doi.org/10.1080/10934529.2012.717811>.
- U.S. Environmental Protection Agency, Guidelines Establishing Test Procedures for the Analysis of Pollutants (App. B, Part 136, Definition and Procedures for the Determination of Detection Limit Method); U.S. Code of Federal Regulations, Title 40, revised July 1, p. 265 (1997).
- P. Devi, M. Kaur, S. Chatterji and R. Thakur, International Conference on Recent Advances and Trends in Electrical Engineering (RATEE-2014) p. 167 (2014).
- S. Hu, J. Lu and C. Jing, *J. Environ. Sci. (China)*, **24**, 1341 (2012); [https://doi.org/10.1016/S1001-0742\(11\)60922-4](https://doi.org/10.1016/S1001-0742(11)60922-4).
- J. Das and P. Sarkar, *Environ. Sci. Water Res. Technol.*, **2**, 693 (2016); <https://doi.org/10.1039/C5EW00276A>.
- M. Salman, M. Athar, Waheed-uz-Zaman, U. Shafique, J. Anwar, R. Rehman, S. Ameer and M. Azeem, *Anal. Methods*, **4**, 242 (2012); <https://doi.org/10.1039/c1ay05569k>.
- K. Hagiwara, Y. Koike, M. Aizawa and T. Nakamura, *Talanta*, **144**, 788 (2015); <https://doi.org/10.1016/j.talanta.2015.07.002>.