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Rapid Quantitative Determination of Total Inorganic Arsenic

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Abstract: Arsenic contamination in the environment has become the worldwide problem due to its harmful effect to the environment. The main species of arsenic found in natural waters are arsenic (III) and arsenic (V). However, arsenic is normally detected as arsenic (III) or arsenic (V), thus, detection of both species in water samples is crucial. Although, colorimetric method is widely used for detection of arsenic on sites but the interpretation of the results is operator dependent. Hence, the main objective of this study is to develop a mathematical model to determine total inorganic arsenic quantitatively where the accuracy and precision of the colorimetric method is enhanced by image processing technique. The developed mathematical model relates total inorganic arsenic concentrations with Red, Green and Blue (RGB) color values and it can be expressed as: total inorganic arsenic = 405-0.133 Red-1.88 Green-0.559 Blue. The adequacy of the model was proved statistically and it can determine total inorganic arsenic with a linear range of 0-300 μg/L and a detection limit of 2.08 μg/L was determined which was lower than previous developed visual method. In addition, less toxic color generating reagent was applied in this study compared to Gutzeit method.

Key words: Crucial, colorimetric, image, Gutzeit method, toxic color, values

INTRODUCTION

Arsenic contamination in the environment from both anthropogenic and natural sources has become the worldwide problem (Nriagu *et al*., 2007) as it has been classified as a class I human carcinogen by the International Agency of Research on Cancer (IARC) Martinez *et al*. (2011). Arsenic can be found in natural waters in a various forms, mainly as inorganic trivalent arsenite (As (III)) and pentavalent arsenate (As (V)) (Lenoble *et al*., 2003). However, lack of study on total inorganic arsenic detection as arsenic is normally detected as arsenic (III) or arsenic (V), individually, hence, detection of both species is crucial.

Numerous field test kits have been developed based on modification of Gutzeit's method and extensively used for determining arsenic semi-quantitatively. However, the results are interpreted manually with naked eyes which can cause false negative or false positive results. To minimise the errors of the interpretations, therefore, there is a need to improve the detection accuracy with assistance of a digital color recording device such as a digital camera. In one of our recent publications, we documented detection of As (III) in synthetic solutions and spiked tap water samples by colorimetric method (Ong *et al*., 2015). Then later the method was further enhanced by incorporating with image processing to detect As (V) by digitising the images of the color compounds formed (Leong *et al*., 2016). Thus, this study presents detection of total inorganic arsenic (both As (III) and As (V)) quantitatively using similar method. For quantification of total inorganic arsenic, a mathematical

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model was developed by multiple regression analysis and various concentrations of synthetic total inorganic arsenic solutions were used to evaluate the accuracy and precision of the model.

MATERIALS AND METHODS

Reagents and apparatus: An arsenic (III) stock solution 1,000 mg As (III)/L, sulfamic acid, sulphuric acid and silver nitrate were purchased from Merck (Germany). Sodium dibasic arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) was obtained from Sigma-Aldrich to prepare stock solution of As (V). About 60 mL of polypropylene bottles were used as reaction bottles. All reagents used were of analytical grade. Ultrapure water (resistivity of 18.2 MΩ.cm) was obtained from Milli-Q purification system (Millipore) and used to rinse glassware, prepare and dilute all solutions. Whatman filter No. 3 was used to prepare silver nitrate-impregnated filter paper. A digital camera (Sony Cyber-shot, DSC-W610) was employed to capture the images of color compounds formed on the silver nitrate-impregnated filter paper.

Arsenic (III) and arsenic (V) stock solutions: Arsenic (III) stock solution containing 1,000 mg As (III)/L was used in this study. A stock solution of arsenic (V) (1000 mg/L) was prepared by dissolving correct amount of sodium dibasic arsenate heptahydrate in 5 mL of 1 N sulphuric acid and then made up to 100 mL with the ultrapure water.

Total inorganic arsenic working standard solutions: Various concentrations (0-600 μg/L) of arsenic (III) and arsenic (V) working standard solutions were freshly prepared by diluting the above stock solutions, respectively, using ultrapure water. Various concentrations of total inorganic arsenic working standard solutions were prepared by mixing 25 mL of desired concentrations of arsenic (III) and arsenic (V) working standard solutions in 50 mL of volumetric flask, respectively with a concentration ratio of 1:1 to obtain final total inorganic arsenic of 0, 10, 50, 100, 200 and 300 μg/L.

Preparation of 5% (w/v) silver nitrate solution: An amount of 5 g of silver nitrate was added into a 100 mL volumetric flask which contained ultrapure water and then dissolved and made up with the ultrapure water.

Preparation of silver nitrate-impregnated filter paper: Silver nitrate-impregnated filter paper, act as an arsine sensor paper and was prepared by immersing a 2.5 cm in diameter round-shaped piece into 5% (w/v) of silver nitrate solution for 2 sec. Then, it was pressed between two pieces of dry Whatman filter papers using a 100 weight load for 20 sec.

Experimental procedure

Colorimetric detection of total inorganic arsenic: Appropriate amount of sulfamic acid was added into 50 mL of total inorganic arsenic working standard solution in a 60 mL of polypropylene bottle. The mixture was swirled before adding zinc powder and swirled again to homogenise the mixture. Then the bottle was closed with a cap which its inner part was covered by the silver nitrate-impregnated filter paper. The mixture was allowed to react for a certain period at 25°C. The impregnated filter paper was removed from the cap when the reaction period was achieved. The experiments were conducted with various mass ratios of sulfamic acid to zinc powder (MSZ) and different Reaction Period (RP) which followed the experimental conditions as presented in Table 1.

Table 1: Design matrix for detection of total arsenic

Std. order	Run order	Pt type	Blocks	MSZ	RP (min)
1	74	1	1	1	5
14	58	1	2	1	5
27	48	1	3	1	5
40	5	1	4	1	5
53	25	1	5	1	5
66	31	1	6	1	5
5	78	-1	1	1	7.5
18	65	-1	2	1	7.5
31	43	-1	3	1	7.5
44	4	-1	4	1	7.5
57	16	-1	5	1	7.5
70	39	-1	6	1	7.5
3	66	1	1	1	10
16	64	1	2	1	10
29	45	1	3	1	10
42	1	1	4	1	10
55	18	1	5	1	10
68	37	1	6	1	10
7	76	-1	1	2.5	5
20	59	-1	2	2.5	5
33	40	-1	3	2.5	5
46	11	-1	4	2.5	5
59	19	-1	5	2.5	5
72	33	-1	6	2.5	5
10	67	0	1	2.5	7.5
13	69	0	1	2.5	7.5
11	71	0	1	2.5	7.5
12	75	0	1	2.5	7.5
9	77	0	1	2.5	7.5
23	53	0	2	2.5	7.5
22	54	0	2	2.5	7.5
24	55	0	2	2.5	7.5
25	60	0	2	2.5	7.5
26	62	0	2	2.5	7.5
36	46	0	3	2.5	7.5
38	47	0	3	2.5	7.5
35	49	0	3	2.5	7.5
39	50	0	3	2.5	7.5
37	51	0	3	2.5	7.5

Table 1: Continue

Std. order	Run order	Pt type	Blocks	MSZ	RP (min)
50	8	0	4	2.5	7.5
51	9	0	4	2.5	7.5
48	10	0	4	2.5	7.5
49	12	0	4	2.5	7.5
52	13	0	4	2.5	7.5
61	14	0	5	2.5	7.5
64	15	0	5	2.5	7.5
62	17	0	5	2.5	7.5
63	20	0	5	2.5	7.5
65	21	0	5	2.5	7.5
75	27	0	6	2.5	7.5
76	28	0	6	2.5	7.5
74	29	0	6	2.5	7.5
77	36	0	6	2.5	7.5
78	38	0	6	2.5	7.5
8	73	-1	1	2.5	10
21	63	-1	2	2.5	10
34	41	-1	3	2.5	10
47	3	-1	4	2.5	10
60	23	-1	5	2.5	10
73	30	-1	6	2.5	10
2	70	1	1	4	5
15	57	1	2	4	5
28	44	1	3	4	5
41	7	1	4	4	5
54	26	1	5	4	5
67	35	1	6	4	5
6	68	-1	1	4	7.5
19	56	-1	2	4	7.5
32	52	-1	3	4	7.5
45	6	-1	4	4	7.5
58	24	-1	5	4	7.5
71	34	-1	6	4	7.5
4	72	1	1	4	10
17	61	1	2	4	10
30	42	1	3	4	10
43	2	1	4	4	10
56	22	1	5	4	10
69	32	1	6	4	10

Quantification of digital image: A digital camera (Sony Cyber-shot, DSC-W610) was employed to record the color image images of the color compounds formed at a fixed distance of 15 cm. The conditions used for capturing images were kept constant including lighting conditions (automatic mode) and camera setting. The images were cropped before transformed into Red, Green and Blue (RGB) digital values by a software, image J. Each color has a digital value which range from 0-225. These digital values were used for mathematical model development and statistical analysis.

Validation of optimum conditions: The experimental data in digital values was analysed by optimisation plot using Minitab Software (Version 17) (US). The optimum conditions of the detection was proposed by the software. Then validation of the optimum conditions was performed by conducting the experiments at the suggested optimum conditions.

Development of mathematical model: To develop a mathematical model for determination of total inorganic arsenic, detection of 0-300 μg/L of total inorganic arsenic working standard solutions were conducted at the optimum conditions in 5 replications using similar procedures as mentioned in the sections of colorimetric detection of total inorganic arsenic and quantification of digital image. The Minitab Software (Version 17) (US) was utilized for data analysis and development of mathematical model. Multiple regression analysis was employed to develop a mathematical model which relates RGB color values to total inorganic arsenic concentrations using general form of multiple regression model as shown in Eq. 1:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 +, \ldots, \varepsilon \quad (1)$$

Where:

x_1-x_3 = The independent variables
Y = The dependent variable
β_0-β_3 = The correlation coefficients
ε = The error term

To evaluate the performance of the developed model, the goodness-of-fit of the developed model was evaluated.

Validation of the developed mathematical model: Validation of the developed model was performed using 10-300 μg/L of total inorganic arsenic based on the procedure as mentioned above. The experiments were performed at the optimum conditions in 5 replications. The results obtained by the developed model were compared with that of the known arsenic (III) concentrations and analysed by paired sample t-test using Minitab Software Version 17.0 (US). The accuracy and precision of the model were examined by Relative Bias (RB) and Relative Standard Deviation (RSD), respectively.

RESULTS AND DISCUSSION

Validation of the optimum conditions was conducted based on the optimum conditions suggested by the optimization plot as shown in Fig. 1. As it can be observed that the optimum conditions for detection of total inorganic arsenic were suggested to be 1.50 g of sulfamic acid and 5.0 min of reaction period in order to obtain approximate color values of R (114.0), G (118.0) and B (95.0) with desirability of 0.9940. Consequently, the suggested optimum conditions were validated by conducting the experiments at these conditions. Experimental and predicted values of response (RGB color values) are presented in Table 2. The experimentally determined R, G and B values were 110.50, 118.30 and 86.10, respectively. It was observed that the experimental determined RGB values are close to that of the model predicted RGB values (R = 113.2857, G = 117.5937 and B = 94.8336) which shows the adequacy of the model. Therefore, it can be concluded that the model can predict RGB color values very well.

Development of mathematical model: Detection of total inorganic arsenic (0-300 μg/L) were conducted at the optimum conditions to develop a mathematical model to determine total inorganic arsenic concentration. The results are expressed in Red, Green and Blue (RGB) color values which range from 0-255 after digitisation the images by Image J. The Standard Deviations (SDs), Relative Standard Deviations (RSDs) and mean of each RGB color value are presented in Table 3. The SDs and RSDs of R ranged from 1.12-2.30 and 0.99-2.60%, respectively while the SDs and RSDs of G were found to be 1.36-2.50 and 1.05-3.57%, respectively. Meanwhile the SDs and RSDs of B were 1.03-2.22 and 1.28-4.33%, respectively. In general, the SDs and RSD were low as indicated by the SDs of RGB were <3 and their RSDs were <5%. After performing multiple regression analysis, the final fitted model can be expressed as shown in Eq. 2:

$$= 405 - 0.133\,\text{Red} - 1.88\,\text{Green} - 0.559\,\text{Blue} \tag{2}$$

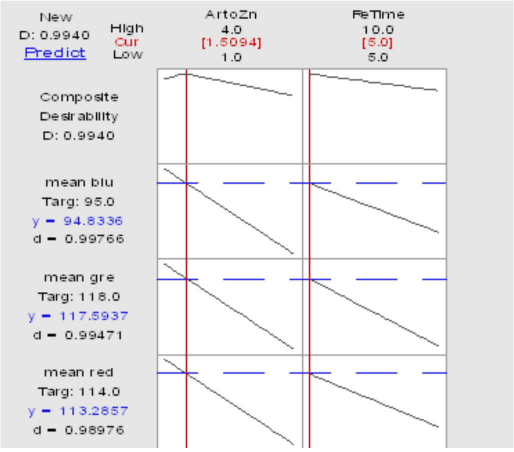

Fig. 1: Optimization plot for detection of total inorganic arsenic

It can be said that the model can be highly fitted by total inorganic arsenic concentrations as indicated by the high coefficient of determination ($R^2 = 0.9574$).

Accuracy and precision of developed mathematical model: The accuracy of a mathematical model is important and hence, the accuracy of the mathematical model developed in this research was evaluated by Relative Bias (RB) as expressed in Eq. 3 whereas the precision of the developed model was also examined by replicating analysis of the color images and RGB calculated from the same sample, and the results are evaluated in terms of RSDs:

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

Where:

d = The difference between the calculated mean of total arsenic (μg/L) and the reference concentration of total arsenic (μg/L)

C_R = The reference concentration of total arsenic (μg/L). The known concentrations of total arsenic were employed as the reference concentrations

Table 4 presents the results of both RBs and RSDs of 5 replications for detection of 10-300 μg/L of total inorganic arsenic. As, it can be observed, the RBs of the calculated total inorganic arsenic concentrations were relative low as indicated by RBs ranged from 0.1-2.8%. Meanwhile, the RSDs were calculated to be <5%, showing good repeatability of the proposed method. Thus, it can be said that the mathematical model could estimate total inorganic arsenic concentrations accurately and precisely. Figure 2 shows a good correlation between known total inorganic arsenic concentration and calculated mean total inorganic arsenic concentrations

Table 2: Experimental and predicted color values at the optimum conditions

	Color values	
Color	Experimental	Predicted
R	110.50	113.29
G	118.30	117.59
B	86.10	94.83

Table 3: Color values of various concentrations of known total inorganic arsenic

Known total inorganic arsenic (μg/L)	Mean of color values			RSD (%)		
	R	G	B	R	G	B
0	148.45±1.30	159.57±1.50	152.52±1.80	0.99	1.05	1.28
10	141.73±2.05	158.39±2.50	140.21±2.22	1.86	2.11	2.31
50	132.02±2.30	142.94±2.06	123.03±2.03	2.60	2.23	3.03
100	121.74±1.90	124.66±1.98	96.10±1.06	2.51	2.52	2.09
200	78.84±1.50	86.29±1.36	56.12±1.28	2.46	2.59	3.64
300	58.79±1.12	45.04±1.62	21.59±1.03	2.11	3.57	4.33

Table 4: Comparison between known total inorganic arsenic concentrations and calculated mean total inorganic arsenic

Knowntotal inorganic arsenic concentration (µg/L)	Calculated mean total inorganic arsenic concentration (µg/L)	RSD (%)	RB (%)
10	10.21±0.4020	3.7	2.1
50	51.17±2.0230	4.0	2.3
100	100.14±4.520	4.5	0.1
200	205.52±7.367	3.6	2.8
300	299.40±1.585	0.5	0.2

Table 5: Mean of calculated total inorganic arsenic and mean recovery of 10 µg/L of total inorganic arsenic

Variables	Values
Mean of calculated total inorganic arsenic±SD (µg/L)	9.967±0.664
Mean of recovery (%)	99.67

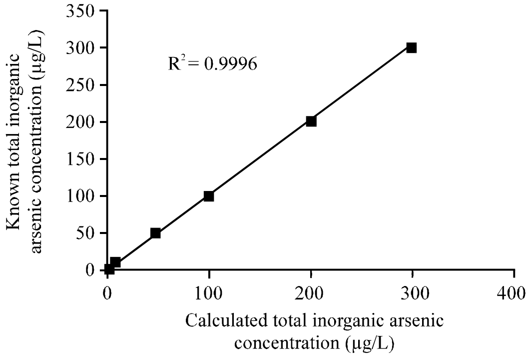

Fig. 2: Correlation between known total inorganic arsenic and calculated total inorganic arsenic

as coefficient of determination, R^2 (0.9996) is high. Consequently, it can be concluded that the model can fit total inorganic arsenic concentrations very well.

Method Detection Limit (MDL): In this study, seven replications of experiment were conducted to detect 10 µg/L of total inorganic arsenic in order to determine Method Detection Limit (MDL). Table 5 shows the experimental results used to determine MDL.

The mean of 10 µg/L of total inorganic arsenic (9.967 µg/L) was used as the estimated MDL due to high recovery (99.67%) and low standard deviation (0.664). Thus, MDL was calculated by multiplying 3.14 with the Standard Deviation (SD) of 10 µg/L of total inorganic arsenic based on the Eq. 4 (Anderson *et al.*, 2008; Rajakovic *et al.*, 2012):

$$\text{MDL} = t \times \text{SD} \tag{4}$$

where, t = 3.14 is the student's t value at 99% confidence level with n = 7 and SD is standard deviation of the replicate samples.

From the calculation, MDL was found to be 2.08 µg/L which was lower than that of some methods/field kits developed by previous researchers. For example, MDL of 5 µg/L was reported by Okazaki *et al.* (2015) using sensitive on-site visual method via molybdenum blue colorimetry combined with membrane filtration technique. The detection limit of field kit developed by Devi *et al.* was 4 µg/L using Gutzeit method. Meanwhile, the detection limit of 8 µg/L was reported by Shan *et al.* in detection of arsenic (III) and arsenic (V) by the formation of molybdenum blue complexes. Das *et al.* (2014) documented that detection limit of arsenic was 10 µg/L by the generation of colour compound from the reaction of arsine produced with either mercuric bromide or silver nitrate without image processing is 10 µg/L. Thus, with the assistance of image processing technique, the MDL found in this study is lower than those without image processing as documented by the previous researcher.

CONCLUSION

A simple and rapid method for quantification of total inorganic arsenic in aqueous solutions was proposed in this research. The detection of total inorganic arsenic was optimised using optimization plot. A mathematical model which relates to total inorganic arsenic concentrations was developed and validated. The results of this study confirm that it is possible to detect total inorganic arsenic accurately and precisely using a combination of colorimetric and image processing technique with the accuracy better than 2.8% and precision <5%.

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