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Improvement of Colorimetric Arsenic (V) Detection by an Image Processing Technique: Application of Factorial Design Analysis

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Abstract : Colorimetric method of commercial test kits for arsenic detection interpreted by visual comparison with standard color chart are normally inaccurate and lack of sensitivity. This paper describes the results of our attempt to improve the reliability of a colorimetric technique to detect arsenic (V) on-site by incorporating an image processing technique into it where various factors that may influence the detection and the optimum conditions for detection were determined. Detection of arsenic (V) was conducted by adding zinc powder into arsenic (V) working solutions under acidic conditions where arsenic (V) is converted to arsine gas and eventually reacts with silver nitrate on the silver nitrate-impregnated filter paper, which forms colored compound. The images of the colored compounds were digitised into Red, Blue, and Green (RBG) color values. The most significant factor was 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, which significantly influence RGB color values and hence influence detection of arsenic (V). The optimum mass ratio of sulfamic acid to zinc powder and reaction period to detect arsenic (V) were found to be 3.0 g: 1.5 g and 5 minutes, respectively, using 100 g of weight load for drying silver nitrate-impregnated filter paper in 20 seconds. Incorporation of image processing into colorimetric method was found to be a more effective technique for detection of arsenic (V).
Keywords : Arsenic (V); Colorimetric; Factorial design of analysis; Image processing; Optimisation.

Introduction

Arsenic is one of heavy metals that are associated with the main threats to human health[1]. This metal is reactive and can easily react with other metallic and non-metallic elements to form oxides, hydrides, sulfides, arsenates, etc[2]. Arsenic compounds are widely found in the environment produced by natural processes and human activities[3]. Chronic consumption of arsenic contaminated water causes skin lesions, neurological disorders and cancers, including cancer of the kidneys and lungs[4]. The current drinking standard set by the US Environmental Protection Agency (EPA) and the World Health Organization (WHO) is 10 µg/L[5]. Instrumental methods such as Inductively Coupled Plasma (ICP-MS) have been developed to quantify trace arsenic[6] but these methods are bulky, expensive and too sophiscated to be used in field analyses.

However, most of the evaluations based on Gutzeit method-based field test kits reported that the test kits are unreliable for detection of arsenic at the concentration close to arsenic toxicity concentration threshold[7]. It appears that the determination of this element at low level by test kits is often difficult due to insufficient sensitivity and selectivity of the test kits.

It was reported that accuracy of arsenic detection using (Macherey–Nagel Quantofix) Arsen10 ® and Arsen 50 ® arsenic test kits increases significantly after developing a new operator-independent analysis which consisted of digitalizing the images of the color formed on the paper strips and performing image analysis[8]. 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 ± 6.2 µg/L with a 95% confidence. However, chemicals used are concentrated hydrochloric acid and mercuric (II) bromide, which are difficult to handle during transportation and dispose as concentrated HCl is highly corrosive and mercuric (II) bromide is a toxic compound.

Recently, we reported detection of As (III) in synthetic solutions and spiked tap water samples by colorimetric method[9]. Later the detection method was improved by incorporated with image processing technique to quantify As (III) by converting the images of the colored compounds formed into red, green and blue (RGB) color values[10]. Consequently, we applied similar technique to enhance detection of As (V) quantitatively in this work. In this study, arsenic (V) concentrations are quantitatively determined by digitizing the color formed from the reaction of arsine gas generated and silver ion impregnated on the filter paper. A 2^4 full factorial design with a blocking factor was used to investigate the effects of weight load used for drying silver nitrate-impregnated filter paper, drying period of silver nitrate-impregnated filter paper, mass ratio of sulfamic acid to zinc powder and reaction period between arsine gas generated and silver nitrate. Optimisation of arsenic (V) detection was also determined by an optimisation plot.

Experimental

Preparation of arsenic (V) working standard solutions and silver nitrate-impregnated filter paper

An arsenic (V) stock solution (1000 mg/L) was prepared by adding 0.4165 g of sodium arsenate heptahydrate ($Na_2HAsO_4•7H_2O$) (Merck) into 5 ml of 1 N sulphuric acid (H_2SO_4) and made up to 100 ml with ultrapure water (18.2 MΩ.cm) which obtained from Milli-Q water purification system (Millipore). Arsenic (V) working standard solutions of 0, 10, 50, 100, 200 and 300 µg/l were freshly prepared by diluting the stock solution using ultrapure water. A 5% (w/v) silver nitrate solution was prepared by dissolving 5.0 g of silver nitrate ($AgNO_3$) into ultrapure water in a 100 mL volumetric flask. Silver nitrate-impregnated filter paper, an arsine sensor, was prepared from a Whatman filter paper No.3. It was cut into a round-shaped piece with a diameter of 2.5 cm, then immersed into the 5% (w/v) silver nitrate solution for 2 seconds and dried by pressing it between two pieces of the dry Whatman filter papers using 100 or 500 g load for 20 or 60 seconds.

Arsenic (V) colored compound formation

Polypropylene bottles (60 mL) were used as reaction bottles. Into each bottle, 50 mL of arsenic working standard solution of As (V) and, chosen amounts of sulfamic acid and zinc powder were transferred and gently swirled to ensure proper mixing. Each reaction bottle was then closed with its cap which the inside part was covered by the silver nitrate-impregnated filter paper and swirled gently again for the selected reaction period. Each experiment was conducted at 25°C and in duplicates. Immediately after the reaction period was over, the colored silver nitrate-impregnated filter paper was removed from the cap and used in the step of analysis.

Color image processing

The colored silver nitrate-impregnated filter paper was placed on a base and two images were captured by a digital camera (Sony Cyber-shot, DSC-W610) at the distance of 15 cm with lighting in automatic mode and these setting was kept constant during the whole process. The images were imported to a computer and cropped before converting into Red (R), Blue (B), and Green (G) color values by Image J. The R, G, and B values for an image were each assigned a value from 0 to 255. The color values were recorded and used for statistical analysis.

Experimental design

The effect of weight load (100 or 500 g), used for drying silver nitrate-impregnated filter paper (DW), drying period of silver nitrate-impregnated filter paper (DP) (20 or 60 s), mass ratio of sulfamic acid to zinc powder (MSZ) (1.0 g: 0.5 g or 4.0 g: 2.0 g), and reaction period (RP) (5 or 10 minutes) were investigated at low and high levels as presented in Table 1.0. In this study, we used Minitab software version 17.0 (Minitab Inc., PA, USA) to randomize the ninety-six experimental runs with all possible combinations of factors in duplicates according to their high and low levels.

Table 1: Low and high levels of factors

Factor	Low level (-1)	High level (+1)
Weight load used for drying silver nitrate-impregnated filter paper (DW), g	100	500
Drying period of silver nitrate-impregnated filter paper (DP), s	20	60
Mass ratio sulfamic acid to zinc powder (MSZ)	1:0.5	4:2
Reaction period (RP), min	5	10

Statistical Analysis

Minitab software version 17.0 (Minitab Inc., PA, USA) was used for data analysis including linear regression analysis, Analysis of Variance (ANOVA), correlation between response variables to determine the significant factors that affect detection of arsenic (V). Main effects plots were also formed for each color values based on the significant contribution factors. Optimisation of arsenic (V) detection was also conducted by optimisation plot using the software.

Results and Discussion

The experimental results of 2^4 full factorial design with a blocking factor for detection of arsenic (V) are shown in Table 2.

Table 2: **Experimental results for detection of arsenic (V)**

Weight load used for drying silver nitrate-impregnated filter paper (g)	Drying period of silver nitrate-impregnated filter paper (s)	Mass ratio of Sulfamic acid to Zn powder	Reaction period (min)	Arsenic (V) Concentration (μg/L)	Red	Green	Blue	StdOrder	RunOrder	CenterPt
100	20	1	5	0	125.738	143.544	132.7125	1	1	1
100	20	1	5	2.4	125.533	142.967	132.236	2	2	1
100	20	1	5	12	122.224	136.9335	119.147	3	3	1
100	20	1	5	24	121.207	135.3385	118.295	4	4	1
100	20	1	5	48	119.3275	129.6	104.666	5	5	1
100	20	1	5	72	111.114	117.3545	86.3995	6	6	1
100	20	4	5	0	121.8685	138.1535	129.178	7	7	1

100	20	4	5	2.4	119.94	135.029	120.948	8	8	1
100	20	4	5	12	103.63	108.5	69.778	9	9	1
100	20	4	5	24	75.5555	77.5955	36.8305	10	10	1
100	20	4	5	48	78.4325	73.2015	32.041	11	11	1
100	20	4	5	72	63.186	58.996	22.7905	12	12	1
100	20	1	10	0	124.3255	141.2315	135.173	13	13	1
100	20	1	10	2.4	125.2735	141.6125	132.1625	14	14	1
100	20	1	10	12	121.09	128.6795	99.154	15	15	1
100	20	1	10	24	116.9535	125.2005	97.8745	16	16	1
100	20	1	10	48	99.3355	115.617	64.1985	17	17	1
100	20	1	10	72	101.601	97.101	56.6615	18	18	1
100	20	4	10	0	118.525	135.418	125.7415	19	19	1
100	20	4	10	2.4	113.995	128.926	108.4375	20	20	1
100	20	4	10	12	99.472	102.4355	52.8715	21	21	1
100	20	4	10	24	71.222	69.6555	31.5615	22	22	1
100	20	4	10	48	53.358	47.5475	22.921	23	23	1
100	20	4	10	72	41.5635	39.43	22.605	24	24	1
100	60	1	5	0	130.065	147.182	139.806	25	25	1
100	60	1	5	2.4	136.6405	153.7665	147.1975	26	26	1
100	60	1	5	12	123.43	138.761	129.8805	27	27	1
100	60	1	5	24	120.452	133.442	114.3855	28	28	1
100	60	1	5	48	105.986	101.7035	58.0465	29	29	1
100	60	1	5	72	95.3785	85.577	39.869	30	30	1
100	60	4	5	0	124.8325	142.297	137.384	31	31	1
100	60	4	5	2.4	122.9155	139.396	131.6375	32	32	1
100	60	4	5	12	118.7595	128.811	95.967	33	33	1
100	60	4	5	24	94.8	100.4235	58.1415	34	34	1
100	60	4	5	48	76.326	72.972	32.903	35	35	1
100	60	4	5	72	67.199	59.1595	22.717	36	36	1
100	60	1	10	0	130.065	147.182	139.806	37	37	1
100	60	1	10	2.4	136.6405	153.7665	147.1975	38	38	1
100	60	1	10	12	123.43	138.761	129.8805	39	39	1
100	60	1	10	24	120.452	133.442	114.3855	40	40	1
100	60	1	10	48	121.0335	129.6595	102.2735	41	41	1
100	60	1	10	72	118.8455	121.5405	81.984	42	42	1
100	60	4	10	0	119.738	136.0225	128.1285	43	43	1
100	60	4	10	2.4	115.893	130.184	108.7085	44	44	1
100	60	4	10	12	91.475	95.462	48.1855	45	45	1
100	60	4	10	24	86.0925	83.2805	31.498	46	46	1
100	60	4	10	48	53.5125	49.1815	23.853	47	47	1
100	60	4	10	72	41.1445	37.3205	21.8835	48	48	1
500	60	1	10	0	124.8255	142.23	135.687	49	49	1
500	60	1	10	2.4	122.966	140.0345	133.324	50	50	1
500	60	1	10	12	126.3955	145.9795	141.002	51	51	1
500	60	1	10	24	122.442	139.338	131.769	52	52	1
500	60	1	10	48	97.719	89.043	46.0665	53	53	1
500	60	1	10	72	84.5965	72.4355	29.1545	54	54	1
500	60	1	5	0	126.3955	145.9795	141.002	55	55	1
500	60	1	5	2.4	122.442	139.338	131.769	56	56	1
500	60	1	5	12	122.6855	137.899	123.722	57	57	1
500	60	1	5	24	119.1625	130.981	108.477	58	58	1
500	60	1	5	48	118.168	124.328	92.718	59	59	1
500	60	1	5	72	108.095	108.872	67.868	60	60	1
500	20	1	5	0	124.8255	142.23	135.687	61	61	1
500	20	1	5	2.4	122.966	140.0345	133.324	62	62	1

500	20	1	5	12	131.8075	148.508	137.8665	63	63	1
500	20	1	5	24	128.4025	142.9695	128.625	64	64	1
500	20	1	5	48	117.312	125.803	103.6975	65	65	1
500	20	1	5	72	104.6075	113.3065	83.8525	66	66	1
500	20	4	5	0	116.111	135.7135	131.0815	67	67	1
500	20	4	5	2.4	117.3165	133.6695	110.7645	68	68	1
500	20	4	5	12	111.518	119.645	76.885	69	69	1
500	20	4	5	24	86.45	91.325	44.355	70	70	1
500	20	4	5	48	66.3615	62.647	24.8335	71	71	1
500	20	4	5	72	54.878	48.345	19.476	72	72	1
500	20	1	10	0	120.971	140.0025	128.4635	73	73	1
500	20	1	10	2.4	122.5675	138.666	120.2095	74	74	1
500	20	1	10	12	113.562	113.562	91.59	75	75	1
500	20	1	10	24	109.657	118.2085	84.7175	76	76	1
500	20	1	10	48	59.6735	58.5625	29.529	77	77	1
500	20	1	10	72	63.1	57.9785	28.861	78	78	1
500	20	4	10	0	117.767	132.344	123.6025	79	79	1
500	20	4	10	2.4	123.73	136.721	110.3695	80	80	1
500	20	4	10	12	103.546	105.3025	51.5225	81	81	1
500	20	4	10	24	75.082	75.964	26.5475	82	82	1
500	20	4	10	48	50.305	48.1045	21.3735	83	83	1
500	20	4	10	72	38.938	38.8705	25.3765	84	84	1
500	60	4	5	0	118.111	135.181	132.878	85	85	1
500	60	4	5	2.4	119.025	136.86	131.485	86	86	1
500	60	4	5	12	112.265	122.165	89.491	87	87	1
500	60	4	5	24	95.4915	94.796	41.379	88	88	1
500	60	4	5	48	72.9835	68.064	21.955	89	89	1
500	60	4	5	72	66.797	56.7445	17.9235	90	90	1
500	60	4	10	0	113.763	130.7445	121.973	91	91	1
500	60	4	10	2.4	113.0165	124.752	92.6625	92	92	1
500	60	4	10	12	92.938	96.3865	44.2545	93	93	1
500	60	4	10	24	71.4945	66.96	24.5145	94	94	1
500	60	4	10	48	47.0845	42.0655	17.7035	95	95	1
500	60	4	10	72	41.506	36.9425	22.301	96	96	1

The general codified model for 2^4 factorial designs is expressed as in equation (1)

$$\eta = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_4 + A_5X_1X_2 + A_6X_1X_3 + A_7X_1X_4 + A_8X_2X_3 + A_9X_2X_4 + A_{10}X_3X_4 + A_{11}X_1X_2X_3 + A_{12}X_1X_2X_4 + A_{13}X_1X_3X_4 + A_{14}X_2X_3X_4 + A_{15}X_1X_2X_3X_4 \quad (1)$$

where η is color value, A_0 is the global mean and A_i is the regression coefficient corresponding to the main factor effects and interactions.

In this study, the response is color (Red, Green and Blue) (RGB) value and the effect of a factor is defined as the change in response[11], which produced by a change in the level of a factor (weight load used for drying silver nitrate-impregnated filter paper, drying period of silver nitrate-impregnated filter paper, mass ratio of sulfamic acid to zinc powder or reaction period). The effects, regression coefficients, standard errors, T and P of each factor for red, green and red color are displayed in Table 3, 4 and 5, respectively. When the effect of a factor is positive, the color value increases as the factor changed from low to high level and vice versa. The results showed that the main effects of mass ratio of sulfamic acid to zinc powder and reaction period were significant at a 5% of probability level ($p < 0.05$) for all color values, whereas only interaction effect of mass ratio of sulfamic acid to zinc powder-reaction period-drying period of silver nitrate-impregnated filter paper interaction was significant at a 5% of probability level ($p < 0.05$) for all color values as well.

Table 3: Statistical parameters for 2^4 full factorial design of red color value

Term	Effects	Coefficients	Standard Error	T	P
Constant		103.04	1.21	85.49	0.000
DW	-4.33	-2.16	1.21	-1.80	0.077
DP	3.74	1.87	1.21	1.55	0.125
MSZ	-26.91	-13.45	1.21	-11.16	0.000
RP	-10.13	-5.06	1.21	-4.20	0.000
DW x DP	-0.45	-0.23	1.21	-0.19	0.852
DW x MSZ	2.37	1.19	1.21	0.98	0.328
DW x RP	-3.48	-1.74	1.21	-1.44	0.153
DP x MSZ	-1.47	-0.74	1.21	-0.61	0.543
DP x RP	1.74	0.87	1.21	0.72	0.473
MSZ x RP	-2.77	-1.39	1.21	-1.15	0.253
DW x DP x MSZ	-1.61	-0.80	1.21	-0.67	0.507
DW x DP x RP	-0.04	-0.02	1.21	-0.02	0.988
DW x MSZ x RP	4.03	2.02	1.21	1.67	0.098
DP x MSZ x RP	-5.66	-2.83	1.21	-2.35	0.022
DW x DP x MSZ x RP	-1.18	-0.59	1.21	-0.49	0.627

Table 4: Statistical parameters for 2^4 full factorial design of green color value

Term	Effects	Coefficients	Standard Error	T	P
Constant		110.69	1.41	78.26	0.000
DW	-4.85	-2.42	1.41	-1.71	0.091
DP	3.10	1.55	1.41	1.10	0.276
MSZ	-32.64	-16.32	1.41	-11.54	0.000
RP	-12.21	-6.11	1.41	-4.32	0.000
DW x DP	-0.62	-0.31	1.41	-0.22	0.828
DW x MSZ	2.80	1.40	1.41	0.99	0.325
DW x RP	-5.04	-2.52	1.41	-1.78	0.079
DP x MSZ	-1.32	-0.66	1.41	-0.47	0.641
DP x RP	2.96	1.48	1.41	1.05	0.298
MSZ x RP	-2.36	-1.18	1.41	-0.83	0.408
DW x DP x MSZ	-2.58	-1.29	1.41	-0.91	0.365
DW x DP x RP	-0.23	-0.12	1.41	0.08	0.935
DW x MSZ x RP	5.45	2.72	1.41	1.93	0.058
DP x MSZ x RP	-7.36	-3.68	1.41	-2.60	0.011
DW x DP x MSZ x RP	-0.54	-0.27	1.41	-0.19	0.850

Table 5: Statistical parameters for 2^4 full factorial design of blue color value

Term	Effects	Coefficients	Standard Error	T	P
Constant		85.98	1.74	49.28	0.000
DW	-4.72	-2.36	1.74	-1.35	0.180
DP	4.16	2.08	1.74	1.19	0.237
MSZ	-41.06	-20.53	1.74	-11.77	0.000
RP	-13.22	-6.61	1.74	-3.79	0.000
DW x DP	-1.31	-0.65	1.74	-0.38	0.708
DW x MSZ	0.88	0.44	1.74	0.25	0.801
DW x RP	-5.47	-2.74	1.74	-1.57	0.121
DP x MSZ	-1.76	-0.88	1.74	-0.50	0.615
DP x RP	5.28	2.64	1.74	1.51	0.134
MSZ x RP	-0.29	-0.15	1.74	-0.08	0.933

DW x DP x MSZ	-1.73	-0.87	1.74	-0.50	0.621
DW x DP x RP	0.05	0.03	1.74	0.02	0.988
DW x MSZ x RP	5.62	2.81	1.74	1.61	0.111
DP x MSZ x RP	-10.79	-5.39	1.74	-3.09	0.003
DW x DP x MSZ x RP	0.20	0.10	1.74	0.06	0.955

The models that relate the levels of parameters and red, green and blue color values are shown in equations 2, 3 and 4, respectively, by substituting the regression coefficients into equation (1).

Red color value = 103.04 -2.16 X_1 + 1.87 X_2 -13.45 X_3 -5.06X_4 - 0.23 X_1X_2 + 1.19 X_1X_3 -1.74X_1X_4 - 0.74X_2X_3+ 0.87 X_2X_4-1.39 X_3X_4 - 0.80 $X_1X_2X_3$ - 0.02 $X_1X_2X_4$ +2.02$X_1X_3X_4$ -2.83 $X_2X_3X_4$ -0.59 $X_1X_2X_3$ X_4 (2)

Green color value = 110.69 -2.42 X_1 + 1.55 X_2 -16.32 X_3 -6.11X_4 - 0.31 X_1X_2 + 1.40 X_1X_3 -2.52X_1X_4 - 0.66X_2X_3+ 1.48 X_2X_4-1.18 X_3X_4 - 1.29 $X_1X_2X_3$ - 0.12 $X_1X_2X_4$ +2.72$X_1X_3X_4$ -3.68 $X_2X_3X_4$ -0.27 $X_1X_2X_3$ X_4 (3)

Blue color value = 85.98 -2.36 X_1 + 2.08 X_2 -20.53 X_3 -6.61X_4 - 0.65 X_1X_2 + 0.44 X_1X_3 -2.74X_1X_4 - 0.88X_2X_3+ 2.64 X_2X_4-0.15 X_3X_4 - 0.87 $X_1X_2X_3$ + 0.03 $X_1X_2X_4$ +2.81$X_1X_3X_4$ -5.39 $X_2X_3X_4$ -0.10 $X_1X_2X_3$ X_4 (4)

The main effects of the factors for red, green and blue color values are illustrated in Figures 1, 2 and 3. When the effect of a factor is positive, red, green and blue color values changed from high to low levels. In contrast, if the effects are negative, the red, green and blue color values formed for high level of the same factor.

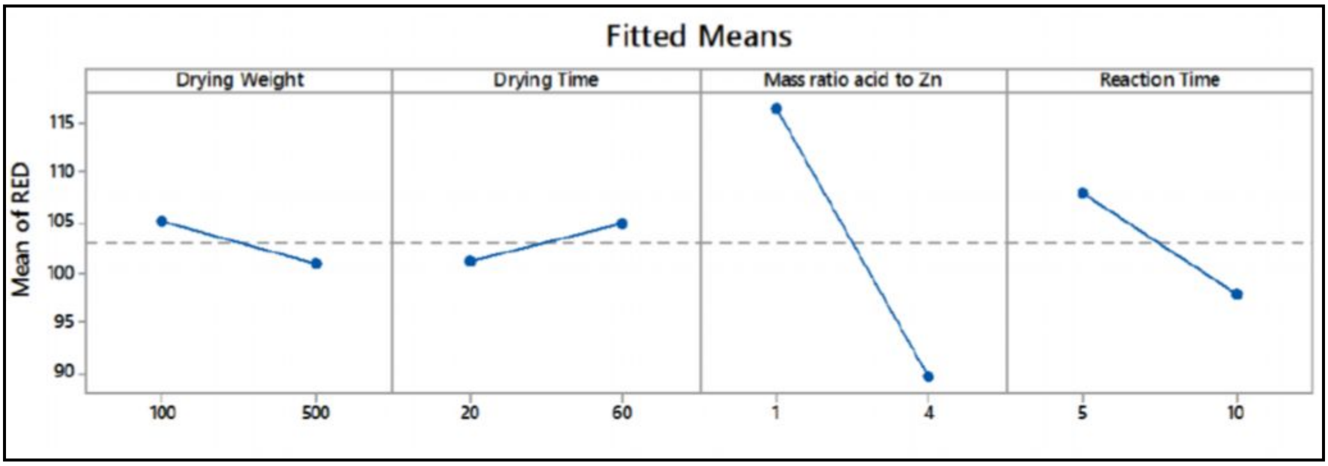

Figure 1. Main effects plot for red color value

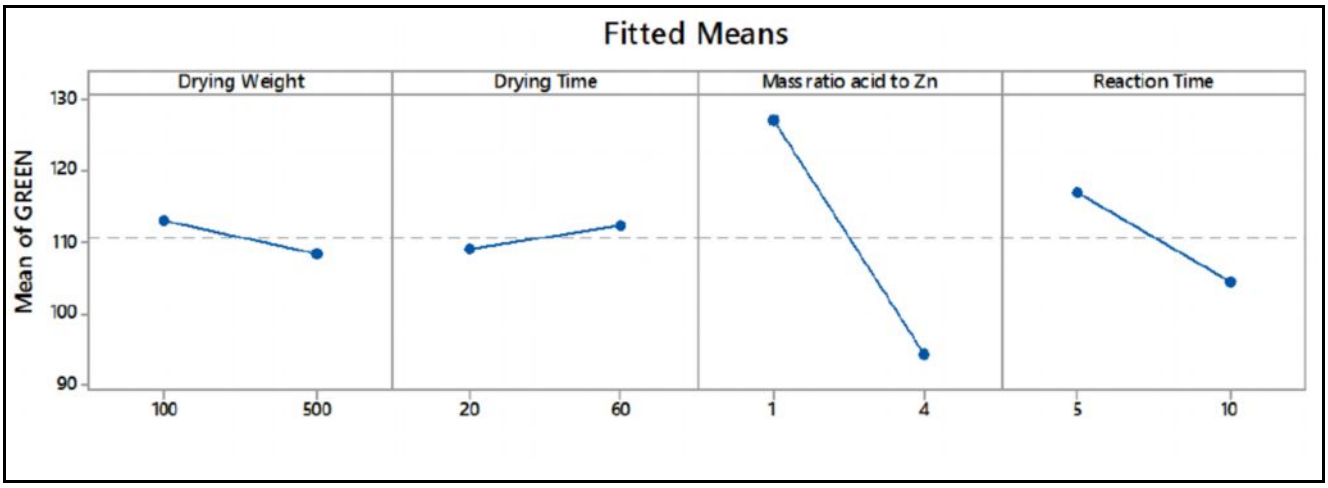

Figure 2. Main effects plot for green color value

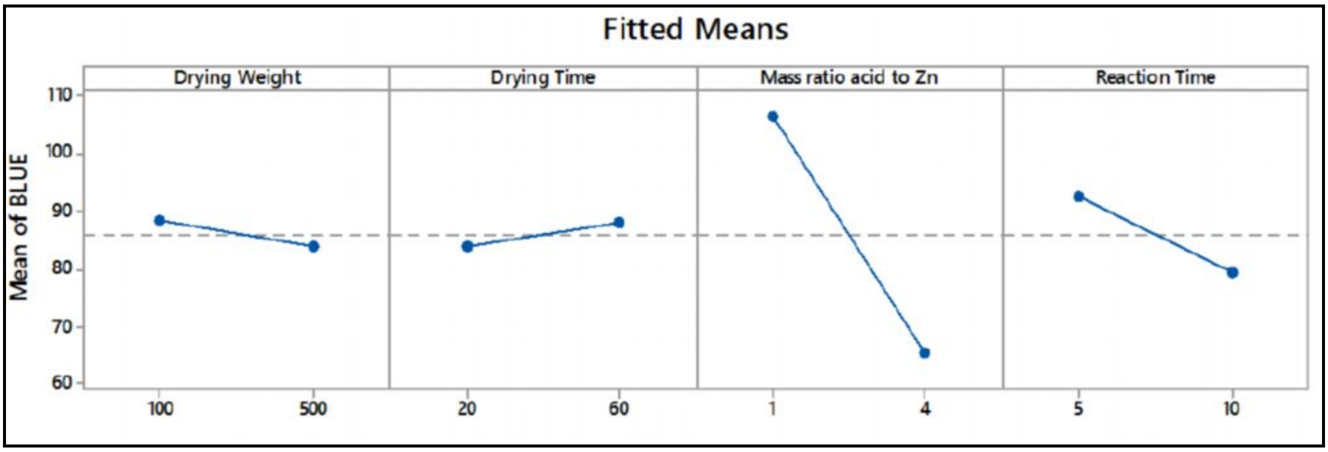

Figure 3. Main effects plot for blue color value

The results of ANOVA for red, green and blue color values are shown in Tables 6, 7 and 8, respectively, whereby the sum of squares used to estimate the factors' effects and F-ratios are also presented. An effect is statistically significant when P-value is less than 0.05.

Table 6: ANOVA of red color value

Term	Degrees of Freedom	Sum of Squares	Mean Square	F	P
BLOCKS	6				
DW	1	449.6	449.6	3.22	0.077
DP	1	335.8	335.8	2.41	0.125
MSZ	1	17376.6	17376.6	124.62	0.000
RP	1	2460.7	2460.7	17.65	0.000
DW x DP	1	4.9	4.9	0.04	0.852
DW x MSZ	1	135.0	135.0	0.97	0.328
DW x RP	1	290.6	290.6	2.08	0.153
DP x MSZ	1	52.1	52.1	0.37	0.543
DP x RP	1	72.4	72.4	0.52	0.473
MSZ x RP	1	184.7	184.7	1.32	0.253
DW x DP x MSZ	1	62.1	62.1	0.45	0.507
DW x DP x RP	1	0.0	0.0	0.00	0.988
DW x MSZ x RP	1	390.7	390.7	2.80	0.098
DP x MSZ x RP	1	768.2	768.2	5.51	0.022
DW x DT x MSZ x RP	1	33.1	33.1	0.24	0.627
Error	75	10457.8	139.4		
Total	96	65524.7			

S = 11.8084 R-sq = 84.04% R-sq(adj) = 79.78%

Table 7: ANOVA of green color value

Term	Degrees of Freedom	Sum of Squares	Mean Square	F	P
BLOCKS	6				
DW	1	564	564.3	2.94	0.091
DP	1	231	230.8	1.20	0.276
MSZ	1	25563	25563.0	133.11	0.000
RP	1	3580	3580.1	18.64	0.000

DW x DP	1	9	9.1	0.05	0.828
DW x MSZ	1	189	188.7	0.98	0.325
DW x RP	1	611	610.8	3.18	0.079
DP x MSZ	1	42	42.1	0.22	0.641
DP x RP	1	211	210.9	1.10	0.298
MSZ x RP	1	133	133.2	0.69	0.408
DW x DP x MSZ	1	159	159.2	0.83	0.365
DW x DP x RP	1	1	1.3	0.01	0.935
DW x MSZ x RP	1	712	712.3	3.71	0.058
DP x MSZ x RP	1	1300	1299.6	6.77	0.011
DW x DP x MSZ x RP	1	7	6.9	0.04	0.850
Error	75	14403	192.0		
Total	96	111967			

S = 13.8580 R-sq = 87.14% R-sq(adj) = 83.71%

Table 8: ANOVA of blue color value

Term	Degrees of Freedom	Sum of Squares	Mean Square	F	P
BLOCKS	6				
DW	1	534	534.0	1.83	0.180
DP	1	416	415.9	1.42	0.237
MSZ	1	40465	40465.2	138.48	0.000
RP	1	4192	4191.7	14.35	0.000
DW x DP	1	41	41.2	0.14	0.708
DW x MSZ	1	19	18.7	0.06	0.801
DW x RP	1	719	719.0	2.46	0.121
DT x MSZ	1	74	74.4	0.25	0.615
DT x RP	1	670	669.6	2.29	0.134
MSZ x RP	1	2	2.1	0.01	0.933
DW x DP x MSZ	1	72	71.9	0.25	0.621
DW x DP x RP	1	0	0.1	0.00	0.988
DW x MSZ x RP	1	759	759.2	2.60	0.111
DP x MSZ x RP	1	2794	2793.6	9.56	0.003
DW x DP x MSZ x RP	1	1	0.9	0.00	0.955
Error	75	21915	292.2		
Total	96	187716			

S = 17.0939 R-sq = 88.33% R-sq(adj) = 85.21%

Some interaction effects are insignificant and eliminated. Hence, recalculation of regression coefficients, standard error, t and p-values were performed with remaining variables and results are presented in Tables 9, 10 and 11 for red, green and blue color values, respectively.

Table 9: Statistical parameters for 2^4 full factorial design of red color value for reduced model

Term	Effects	Coefficients	Standard Error	T	P
Constant		103.04	1.21	85.49	0.000
MSZ	-26.91	-13.45	1.21	-11.16	0.000
RP	-10.13	-5.06	1.21	-4.20	0.000
DP x MSZ x RP	-5.66	-2.83	1.21	-2.35	0.022

Table10: Statistical parameters for 2^4 full factorial design of green color value for reduced model

Term	Effects	Coefficients	Standard Error	T	P
Constant		110.69	1.41	78.26	0.000
MSZ	-32.64	-16.32	1.41	-11.54	0.000
RP	-12.21	-6.11	1.41	-4.32	0.000
DP x MSZ x RP	-7.36	-3.68	1.41	-2.60	0.011

Table 11: Statistical parameters for 2^4 full factorial design of blue color value for reduced model

Term	Effects	Coefficients	Standard Error	T	P
Constant		85.98	1.74	49.28	0.000
MSZ	-41.06	-20.53	1.74	-11.77	0.000
RP	-13.22	-6.61	1.74	-3.79	0.000
DP x MSZ x RP	-10.79	-5.39	1.74	-3.09	0.003

Reduced model equations with resultant coefficients for red, green and blue color values are expressed in equations 5, 6 and 7, respectively.

Red color value $= 103.04 - 13.45\ X - 5.06X_4 - 2.83\ X_2X_3X_4$ (5)
Green color value $= 110.69 - 16.32\ X_3 - 6.11X_4 - 3.68\ X_2X_3X_4$ (6)
Blue color value $= 85.98 - 20.53\ X_3 - 6.61X_4 - 5.39\ X_2X_3X_4$ (7)

Tables 12 to 14 show the results of ANOVA of red, green and blue color values of reduced model, respectively. It can be concluded that mass ratio of sulfamic acid to zinc powder (MSZ) was the strongest effect of the overall contributed to the three color values. The X_3 coefficient was the largest negative coefficient for the three models (5), (6) and (7). The second important factor to the overall optimization was reaction period (RP). The longer the reaction period, three color values decreased accordingly. The interaction of three factors (mass ratio of sulfamic acid to zinc powder (MSZ), reaction period (RP) and drying period of silver nitrate-impregnated filter paper (DP)) are significantly contributed to the three color values was the third significant factor.

Table 12: ANOVA of red color value of reduced model

Term	Degrees of Freedom	Sum of Squares	Mean Square	F	P
BLOCKS	6				
MSZ	1	17376.6	17376.6	121.24	0.000
RP	1	2460.7	2460.7	17.17	0.000
DP x MSZ x RP	1	768.2	768.2	5.36	0.023
Error	87	12468.9	143.3		
Total	96	65524.7			

S = 11.9717 R-*sq* = 80.97% *R-sq(adj)* = 79.22%

Table 13: ANOVA of green color value of reduced model

Term	Degrees of Freedom	Sum of Squares	Mean Square	F	P
BLOCKS	6				
MSZ	1	25563	25563.0	128.75	0.000
RP	1	3580	3580.1	18.03	0.000
DP x MSZ x RP	1	1300	1299.6	6.55	0.012
Error	87	17273	198.5		
Total	96	111967			

S = 14.0905 R-*sq* = 84.57% *R-sq(adj) = 83.15%*

Table 14: ANOVA of blue color value of reduced model

Term	Degrees of Freedom	Sum of Squares	Mean Square	F	P
BLOCKS	6				
MSZ	1	40465	40465.2	139.58	0.000
RP	1	4192	4191.7	14.46	0.000
DP x MSZ x RP	1	2794	2793.6	9.64	0.003
Error	87	25222	289.9		
Total	96	187716			

S = 17.0267 R-*sq* = 86.56% *R-sq(adj)* = *85.33%*

Meanwhile, interaction plots of effects of red, green and color values are illustrated in Figures 4, 5 and 6 respectively. It was observed that the effect of mass ratio of sulfamic acid to zinc powder (MSZ) as reactant was more obvious when the ratio was high with reduction of color values. Similar observation was found for the effect of reaction period (RP).

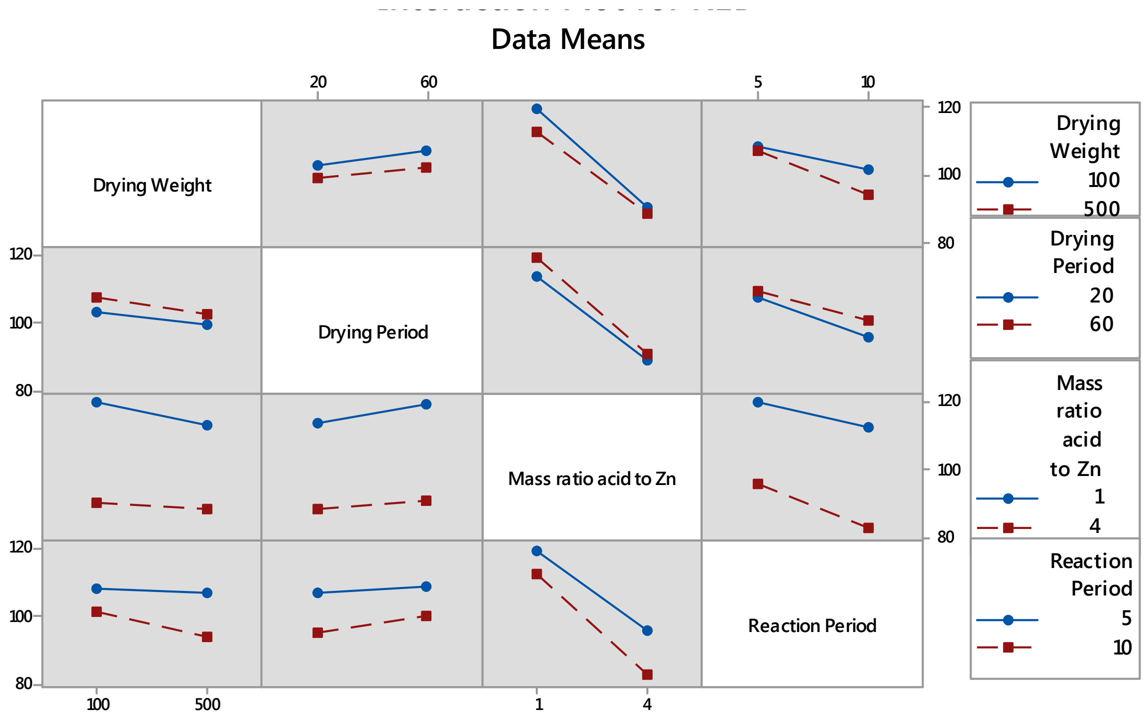

Figure 4. Interaction effects of red color value of reduced model

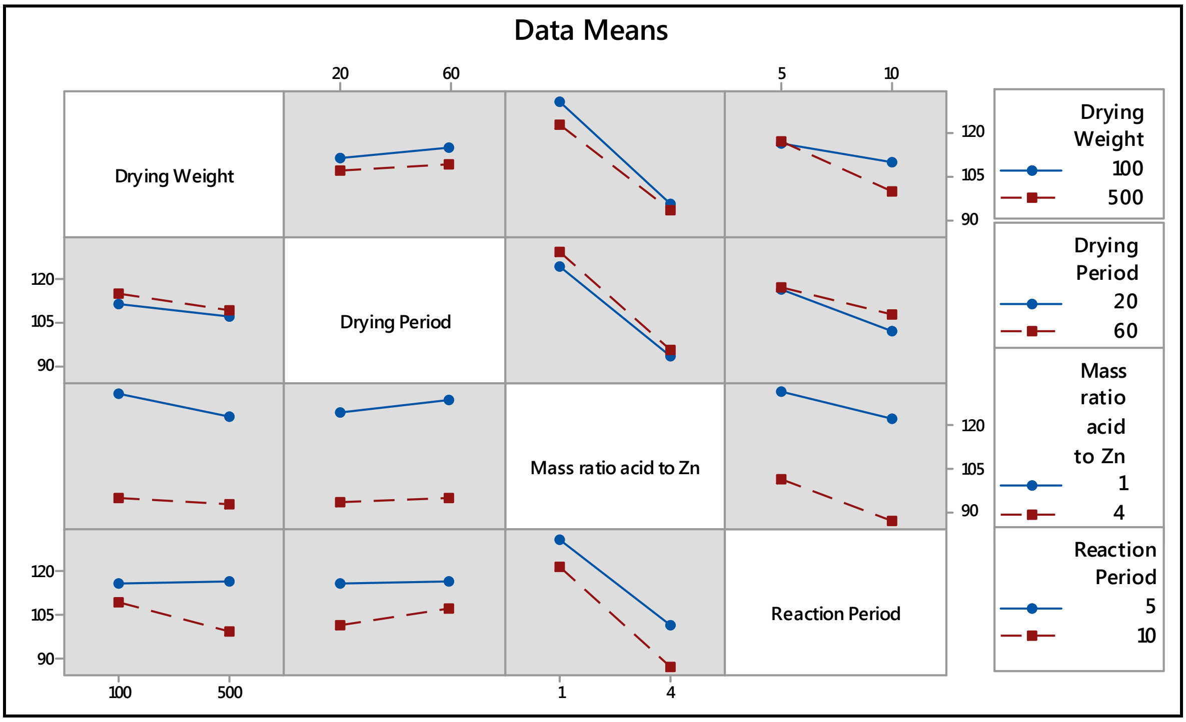

Figure 5. Interaction effects of green color value of reduced model

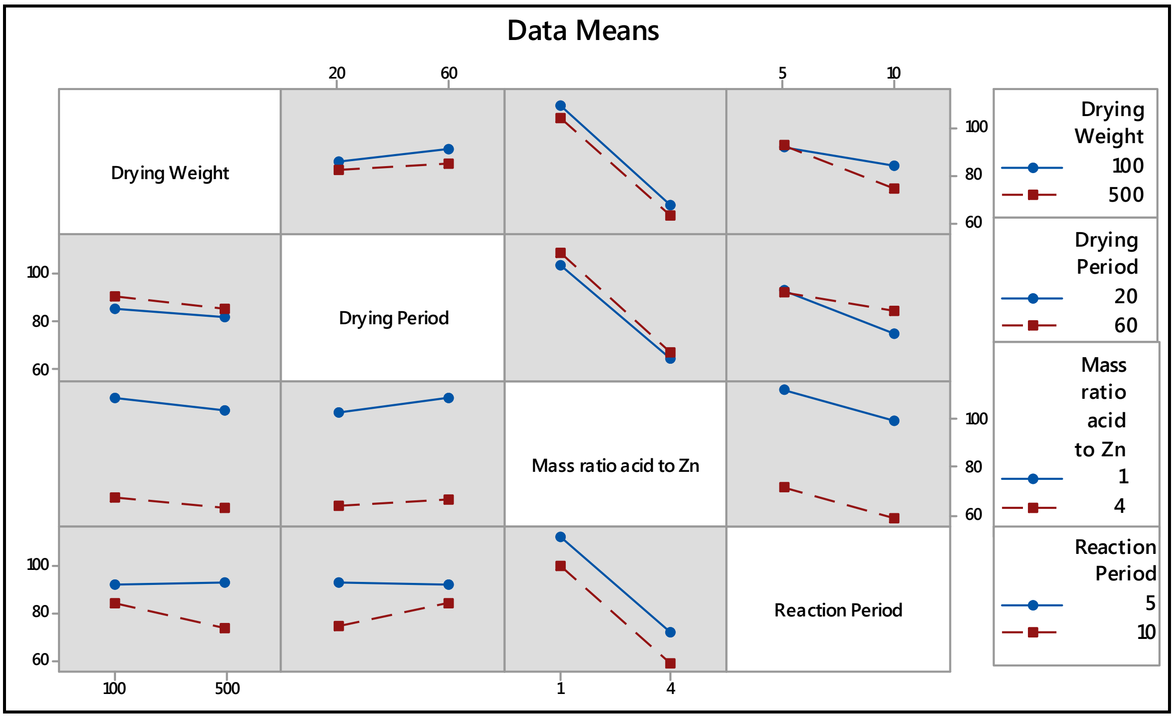

Figure 6. Interaction effects of blue color value of reduced model

The normal probability plots of residual values are depicted in Figures 7, 8 and 9 of red, green and blue color values, respectively, and showed that the experimental data are normal distributed as the experimental points were reasonably aligned.

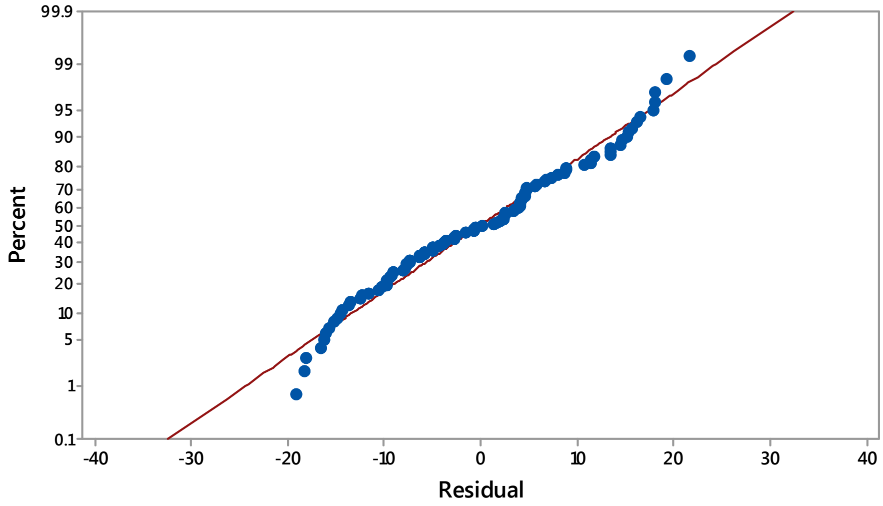

Figure 7. Normal probability plot of residual values of red color value

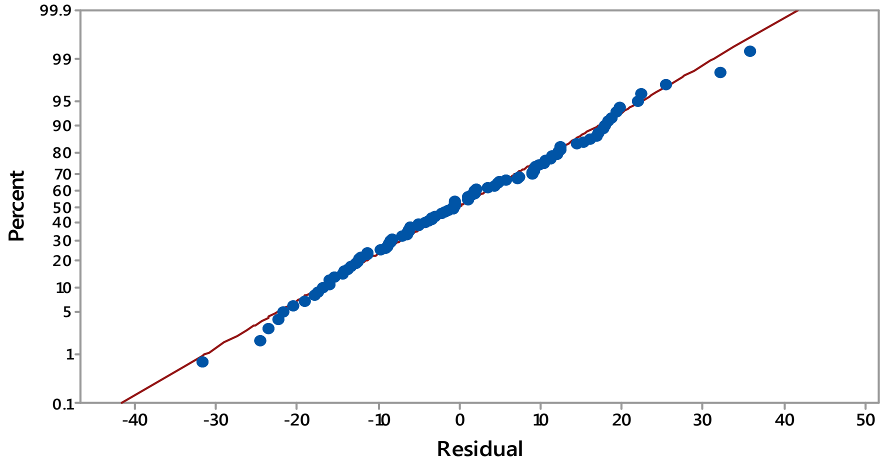

Figure 8. Normal probability plot of residual values of green color value

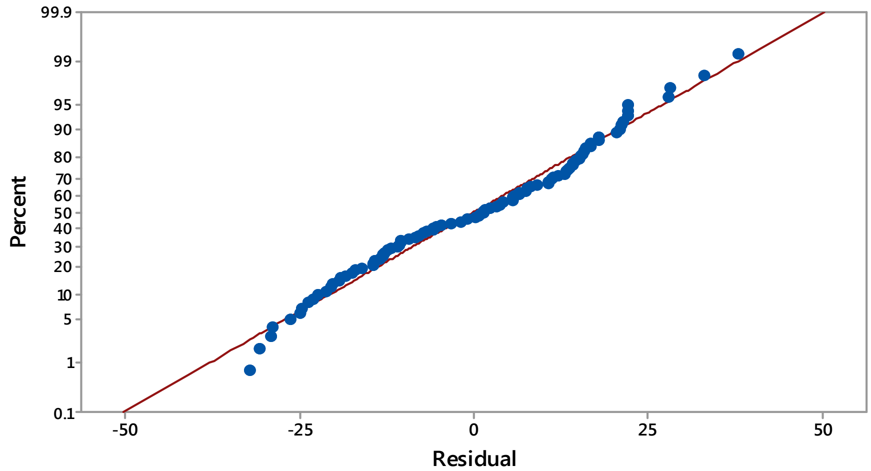

Figure 9. Normal probability plot of residual values of blue color value

Plots of residual versus fitted value of red, green and blue color values are shown in Figures 10, 11 and 12, respectively. All points were found to between the ranges of +20 to -20 for red color value while the ranges for green and blue color values are between +40 to - 40 which clearly showed that there were not outliers.

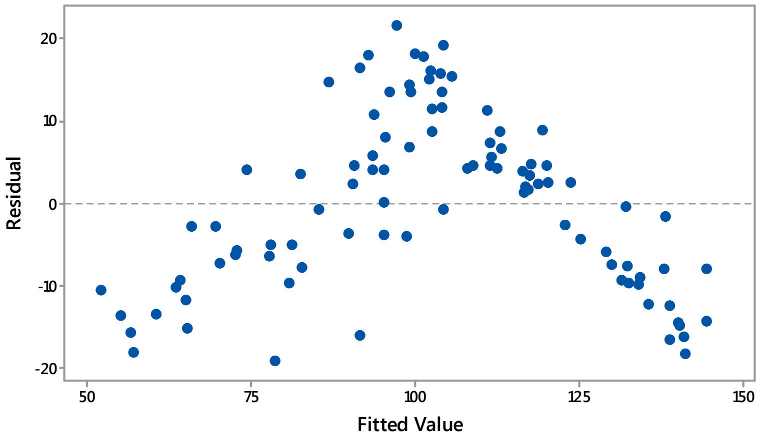

Figure 10. Residual versus fitted value plot of red color value

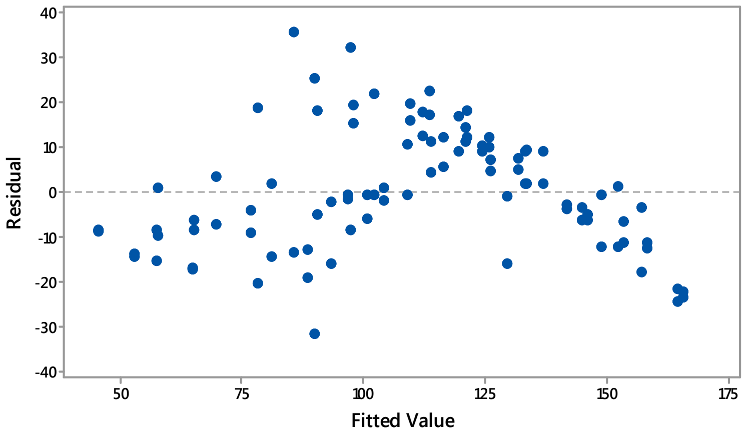

Figure 11. Residual versus fitted value plot of green color value

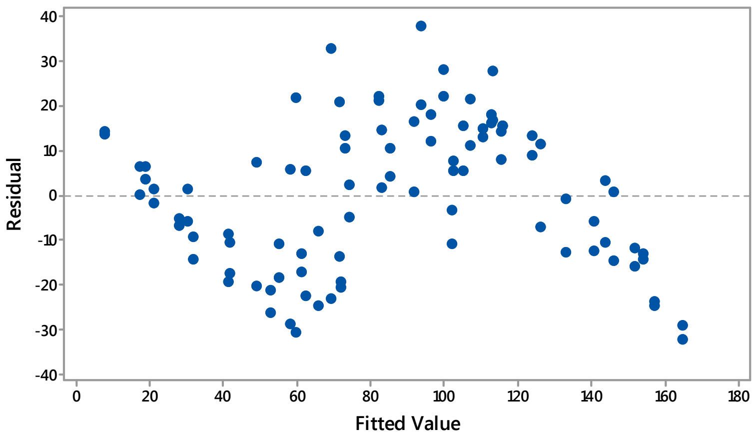

Figure 12. Residual versus fitted value plot of blue color value

Main effect of mass ratio of sulfamic acid to zinc powder

From the above analysis, particularly Tables 9 to 11 clearly show that the mass ratio of sulfamic acid to zinc powder has a significant effect on the color values as indicated by $p < 0.05$. The mass ratio of sulfamic acid to zinc powder is the most significant factor as shown by Figures 7, 8 and 9. An increase in the mass ratio of sulfamic acid to zinc powder from 1 g: 0.5 g to 4 g: 2 g, the RGB values decreased. The highest decreased was observed for blue color value, followed by green, and red color values with 38.55 %, 25.70%, 23.10% (as illustrated in Figures 1-3), respectively, as it was observed that higher mass ratio of sulfamic acid to zinc powder resulted in darker color complex was formed on the silver nitrate impregnated filter paper. This is due to the fact that when higher amount of reagents are added, more arsenic (V) is reduced to arsine gas which reacts with silver nitrate on the impregnated filter paper.

Main effect of reaction period

Apart from effect of mass ratio of sulfamic acid to zinc powder, the reaction period also give significant effect on color values (Tables 9 to 11), as we observed when the reaction period increased from 5 minutes to 10 minutes, red, green and blue values decreased by 9.37%, 10.34% and 14.27%, respectively can be observed from Figures 1 to 3. This can be explained by the fact that as the reaction period increased, more arsenic (V) is reduced to arsine gas which will reacts with silver nitrate to form colored compound on the impregnated filter paper. In fact, darker color has lower color value, hence red, green and blue values decreased when higher reaction period was applied to the reaction.

Interaction effect of mass ratio of sulfamic acid to zinc powder-reaction period-drying period of silver nitrate-impregnated filter paper

Besides main effects, the interaction effect between the parameters can explain better about the reaction. Figures 4, 5 and 6 show two interaction effects between drying weight and drying period; drying weight and mass ratio of sulfamic acid to zinc powder; drying period and reaction period; drying period and mass ratio of sulfamic acid to zinc powder; drying period and reaction period as well as mass ratio of sulfamic acid to zinc powder and reaction period. The results revealed that all two interaction effects are insignificant affect red, green and blue values (Tables 6 to 8). Similar results were observed for three interaction effects and four interaction effects except one of the three interaction effects i.e. mass ratio of sulfamic acid to zinc powder-reaction period-drying period of silver nitrate-impregnated filter paper was significant affect red, green and blue color values on development of colored compound on the silver nitrate-impregnated filter paper. However, such interactions could not be detected using conventional design of experiments.

Optimum of Arsenic (V) Detection

Optimization plot is used to obtain predicted response with higher desirability score, lower-cost factor settings with near optimal properties, to study the sensitivity of response variables to changes in the factor settings and to get required responses for factor settings of interest[12].

A total of 78 experiments were conducted by repeating the experiments with fixed drying period (DP) in 20 seconds and drying weight (DW) of 100 g in combination with mass ratio of sulfamic acid to zinc powder (MSZ) in three different ratios, 1.0 g: 0.5 g; 2.5 g: 1.25 g and 4.0 g: 2.0 g and reaction period (RP) in three different reaction periods (5 minutes, 7.5 minutes and 10 minutes) to validate the optimum conditions by optimum plot using Minitab software version 17.

To achieve approximately digital values for R (130.110), G (138.7960) and B (110.1450), the optimum values of different process parameters were found to be 3.0556 g for sulfamic acid and 5.0 minutes for reaction time with desirability = 0.9875 as illustrated in Figure 13. Experiments were carried out in the conditions as suggested by model. The results revealed that there was good agreement between experimentally determined response factor (R = 130.11, G = 138.80 and B =110.15) and model predicted response factor (R = 130.0304, G = 137.9393 and B =111.4453).

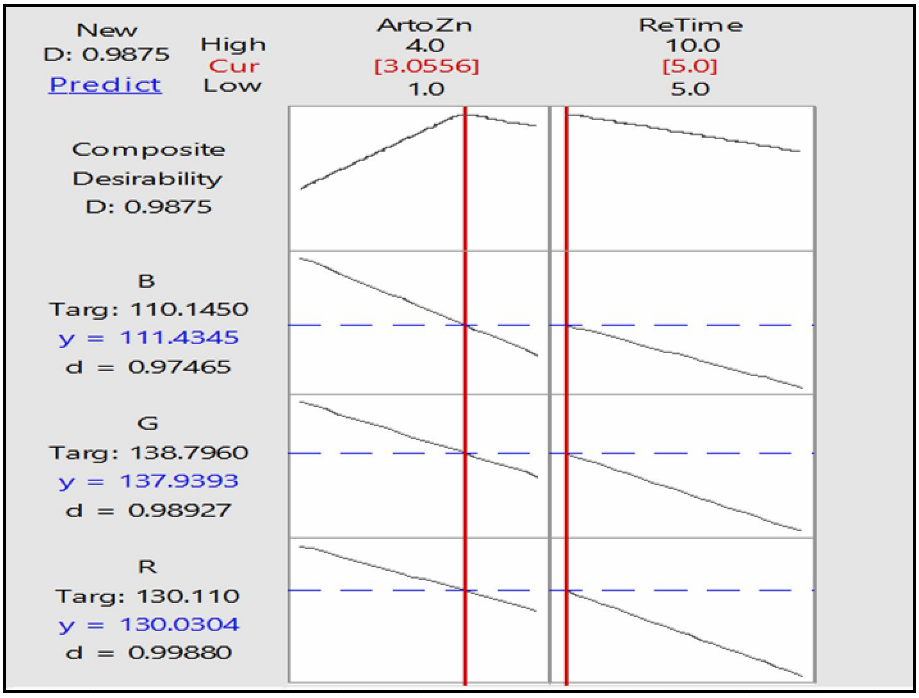

Figure 13. Response optimization plot for the optimum detection of arsenic (V)

In addition, the experimental and predicted RGB values were insignificant different as indicated by *p*-values were higher than 0.05 from the results of Paired Sample t-test as presented in Table 15. Thus, these results can confirm the adequacy of the suggested model.

Table 15. Predicted and experimental RGB color values for arsenic (V) detection

Color	Color Value		*p*-value of Paired Sample *t*-test
	Predicted	Experimental	
R	130.0304	130.11	0.390
G	137.9393	138.80	0.473
B	111.4345	110.15	0.560

The detection time of arsenic (V) found in this study is shorter than that of the test kit reported[13] which requires 7 minutes based on visual determination, 20 minutes with MERCK kit and 30 minutes with Hach Kit. Besides that, ARSENATOR, a commercial digital arsenic test kit requires 20 minutes to detect arsenic in water samples was documented[7]. Hence, it can be said that shorter detection time was found in this study to detect arsenic (V) concentration compared to the commercial test kits.

Conclusion

The effects of weight load used for drying silver nitrate-impregnated filter paper, drying period of silver nitrate-impregnated filter paper, mass ratio of sulfamic acid to zinc powder and reaction period was designed using 2^4 full factorial design for determination of arsenic (V) by image processing assisted colorimetric technique. The results were evaluated by ANOVA, t-test and F-test. It can be concluded that the most significant factor was 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 which significantly affect red, green and blue color values and hence influence detection of arsenic (V). The optimum mass ratio of sulfamic acid to zinc powder and reaction period to detect arsenic (V) were found to be 3.0 g: 1.5 g and 5 minutes, respectively, using 100 g of weight load for drying silver nitrate-impregnated filter paper in 20 seconds.

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