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Design and Development of Aptamer-Gold Nanoparticle for Colorimetric Detection of Methylphosphonic Acid

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Abstract

Response surface methodology (RSM) based on face-centered central composite design (FCCCD) was applied to optimize the methylphosphonic acid (MPA) detection using DNA aptamer-citrate capped-gold nanoparticles (DNA aptamer-cit-AuNPs). Images of the color change of the solution were captured and further processed to red (R), green (G) and blue (B) values. Three parameters: cit-AuNPs concentration (X_1), DNA aptamer concentration (X_2) and incubation period (X_3) were investigated. Analysis of variance (ANOVA) was used to determine the significant factors affecting the detection. Both predicted and experimental responses show good agreement at 95% confidence level. Hence, this method could be implemented for on-site MPA detection.

Keywords: methylphosphonic acid; cit-AuNPs; DNA aptamer

1. Introduction

Organophosphate (OP) nerve agents (NAs) have been used as chemical warfare agents (CWAs) and in agricultural sector as pesticides and insecticides [1]. Nerve agents (NAs) are extremely toxic, inhibiting acetylcholinesterase (AChE) function by binding to the AChE [2]. These NAs hydrolyze the acetylcholine resulting in cholinergic crisis on all organ systems [3]. In environment and human body, NAs can rapidly hydrolysed to alkyl methylphosphonic acid and further hydrolyzed to methylphophonic acid (MPA) [4].

Disposal of CWAs and pesticides into water has raised awareness as it could contaminate our food chain, leading to the development of sensitive, fast, reliable and effective methods in detecting OPs [5]. Gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE) and mass spectrophotometry (MS) [6-10] are the most common analytical techniques applied for detecting OP pesticides as these techniques very sensitive, accurate and reliable. However, these techniques are complicated, expensive, time-consuming and have to be performed by the expert technicians.

The use of colorimetric methods together with metal nanoparticles as a nanoprobe has overcome the problems associated with conventional analytical methods due to its simplicity, fast, portable and inexpensive imaging techniques [11, 12]. Gold nanoparticles (AuNPs) is a kind of noble nanoparticles which can be utilized in designing the colorimetric assay [13]. In the present of analyte ions, the AuNPs were triggered to aggregate and causes red color solution (dispersed state) changes to blue/purple color (aggregate state), which can be visualized by naked eyes or UV-Vis spectrophotometer [14].

Moreover, sensitive nanosensor can be obtained by coupling the aptamer to AuNPs for the detection of target molecules [15]. Aptamer is a single stranded DNA or RNA molecule that binds to the target molecules with high

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specificity and affinity [13, 16]. Binding of aptamer to the target molecule leads to the aggregation of AuNPs which simultaneously affecting the AuNPs stability due to the conformational change of the aptamer [13].

Detection accuracy of an OP was enhanced using a colorimetric sensor array consisting of citrate-capped gold nanoparticles (Cit-AuNPs) which coupled with image processing [17]. The principle of this method is based on the measurement of color characteristics in each pixel which represented by red (R), green (G) and blue (B) as the color changes when the sensors interact with the tested substances [18].

The face-centered central composite design (FCCCD) of the response surface methodology (RSM) is a combination of statistical and mathematical techniques used to reduce the number of experiments for optimization of the variables and responses [19, 20].

Based on the above literatures, the aim of this objective was to develop a simple and rapid method to detect MPA by employing RSM to easily optimize the following parameters: cit-AuNPs concentration, DNA aptamer concentration and incubation period.

2. Materials and methods

The analytical grades of gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) (Sigma Aldrich, USA), tri-sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$) (Merck, Germany), methylphosphonic acid ($CH_3P(O)(OH)_2$) (Merck, Germany) were used. Ultrapure water (18.2 MΩ.cm resistivity, Milli-Q, Millipore) was utilised throughout this study to prepare all solutions and kept at 4°C for further use. Thiolated DNA aptamer with sequence: 5'-/5ThiolMC6-D/ATC CGT CAC ACC TGC TCT CGA TGA GAC AAG AGG AAC ACG GCA CAA TTG ATT TAA TGG TGT TGG CTC CCG TAT-3' was purchased from Integrated DNA Technologies Inc. (Singapore) for detection of methylphosphonic acid, whereas IDTE pH 8.0 (IXTE solution) (10 mM Tris, 0.1 mM EDTA) was also obtained from Integrated DNA Technologies Inc. (Singapore) for resuspension and dilution of the thiolated DNA aptamer.

2.1. Preparation of thiolated DNA aptamer solution

The DNA aptamer used in this study was similar to the DNA aptamer used in the previous study [21] by modification with a thiol group. A stock solution of 841 µL of 100 µM of thiolated DNA aptamer solution was prepared by dissolving the DNA aptamer with IDTE pH 8.0 (IXTE solution) and stored in a freezer at temperature of -20°C.

2.2. Synthesis of citrate-capped gold nanoparticles

Citrate-capped gold nanoparticles (cit-AuNPs) were synthesized based on the reduction of $HAuCl_4$ by citrate as reported by Turkevich et al. [22]. Briefly, 100 mL of 0.2157 mM of $HAuCl_4.3H_2O$ was boiled to 100°C in 250 mL conical flask. After 15 min, 2 mL of 34 mM of tri-sodium citrate dihydrate solution was then added rapidly into the boiling $HAuCl_4.3H_2O$ solution under vigorous stirring (1500 rpm). Subsequently, the mixture was boiled for another 20 min at 1500 rpm. Then, the solution was allowed to cool to room temperature and kept at 4°C for further use in the detection experiments. The concentration of synthesised cit-AuNPs was calculated using Eq. 1 [10]:

$$C_{AuNPs}=6\left(\frac{C_{Au} \times M_{Au}}{\pi \times \rho_{Au} \times D_{AuNPs}{}^{3} \times N_{A}}\right) \quad (1)$$

where C_{AuNPs} is molar concentration of AuNPs (M), C_{Au} is molar concentration of gold atoms/ions (M), M_{Au} is molecular weight of gold ($gmol^{-1}$), ρAu is density of gold (which is 19.3 gcm^{-3}), DAuNPs is diameter of AuNPs (which is 23.5 nm) and NA is Avogadro constant (mol^{-1}).

2.3. Experimental design of colorimetric detection of MPA

In this study, Minitab 16 software (Minitab Inc., USA) was applied for experimental design, mathematical modelling and optimization of DNA aptamer-cit-AuNPs colorimetric assay based on FCCCD of RSM. The independent factors (variables) evaluated were concentration of cit-AuNPs (X_1), concentration of DNA aptamer (X_2) and incubation period (X_3). Table 1 shows the range and levels of each independent variable investigated at three levels: -1, 0 and +1. RSM/FCCCD suggested a total of 20 experimental runs with 8 factorial points, 6 centre points in cube and 6 axial points as shown in Table 2.

Table 1. Parameters, experimental range and level of independent variables used for MPA detection.

Factor		Unit	Low level (-1)	Center level (0)	High level (+1)
X_1	Concentration of cit-AuNPs	nM	0.27	0.35	0.43
X_2	Concentration of DNA aptamer	µM	1	5	9
X_3	Incubation period	min	0	30	60

In this study, DNA aptamer-cit-AuNPs was used as an aptasensor for MPA (15 mM) detection. A volume of cit-AuNPs (500, 650 or 800 µL) and 1 µL of DNA aptamer with different concentrations were mixed in 3 mL screwed cap glass vial and incubated at 37°C for different incubation periods. Then, MPA solution was pipetted into the mixture until the total volume of the mixture reached 1 mL and swirled. After detection period, the mixture was captured using a smartphone at a distance of 9 cm between the camera and the sample in an image capturing box. All conditions including the distance, placement of the sample in the image capturing box and camera setting were kept constant throughout the experiments. All the detection experiments were conducted in triplicates at room temperature.

Table 2. Experimental designs of the three levels independent variables.

Standard order	Run order	Concentration of cit-AuNPs (nM)	Concentration of DNA aptamer (µM)	Incubation period (min)
20	1	0.35	5	30
3	2	0.27	9	0
18	3	0.35	5	30
1	4	0.27	1	0
4	5	0.43	9	0
15	6	0.35	5	30
7	7	0.27	9	60
16	8	0.35	5	30
14	9	0.35	5	60
8	10	0.43	9	60
19	11	0.35	5	30
12	12	0.35	9	30
9	13	0.27	5	30
17	14	0.35	5	30
5	15	0.27	1	60
2	16	0.43	1	0
13	17	0.35	5	0
10	18	0.43	5	30
11	19	0.35	1	30
6	20	0.43	1	60

A second-order polynomial model (Eq. 2) was used to predict the response value over a range of input independent variable values:

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 \qquad (2)$$

where Y is the predicted response (ΔRGB), β_o is the intercept, β_i is the effects of the linear terms, β_{ii} is the effects of the quadratic terms, β_{ij} is the effects of the interaction terms and X_i are the coded value of the corresponding i^{th} factors.

To determine the appropriate model, an analysis of variance (ANOVA) was applied. The fit of the models was evaluated by determining the coefficients (R^2) and adjusted R^2 (*R^2adjusted*).

2.4. Image processing of cropped images

The cropped color images of the mixtures were analysed using ImageJ software to translate the images into red (R), green (G) and blue (B) values. Then, the average R, G, and B values were used to calculate the response (ΔRGB) using Eq. 3 [23]:

$$\Delta \mathrm{RGB} = \sqrt{(R_0 - R_1)^2 + (G_0 - G_1)^2 + (B_0 - B_1)^2} \quad (3)$$

where R_o, G_o and B_o are red, green and blue value of blank, while R_1, G_1 and B_1 are red, green and blue value of MPA.

3. Results and Discussion

3.1. Effect of independent variable on sensing response (ΔRGB)

The effect of three independent variables (concentration of cit-AuNPs, concentration of DNA aptamer and incubation period) on the colorimetric assay were investigated. A total number of 20 experiments design matrix and the sensing responses (ΔRGB) are depicted in Table 3. The obtained results were then subjected to statistical analysis using RSM to evaluate the relationship between the concentration of cit-AuNPs, concentration of DNA aptamer and incubation period.

Table 3. Various experimental runs and the correspondent responses for MPA detection.

Run order	X_1	X_2	X_3	Response	
				Experimental	Predicted
1	0.35	5	30	68	68
2	0.27	9	0	49	49
3	0.35	5	30	68	68
4	0.27	1	0	54	54
5	0.43	9	0	26	26
6	0.35	5	30	69	68
7	0.27	9	60	43	43
8	0.35	5	30	68	68
9	0.35	5	60	60	60
10	0.43	9	60	19	20
11	0.35	5	30	68	68
12	0.35	9	30	62	62
13	0.27	5	30	59	59
14	0.35	5	30	68	68
15	0.27	1	60	49	49
16	0.43	1	0	27	27
17	0.35	5	0	65	65
18	0.43	5	30	34	34
19	0.35	1	30	65	65
20	0.43	1	60	21	21

3.2. Analysis of variance (ANOVA) and model development

In this study, ANOVA was used to examine the significance of the independent variables and their interactions. The responses were evaluated at 95% confidence level (α= 0.05) to determine the adequacy and statistical significance of the second-order polynomial model.

The ANOVA results are presented in Table 4 for each of the model terms. Based on the p-values, the first order effects of all variables (X_1, X_2 and X_3) and two-level interaction of X_1 and X_2 (X_{12}) are significant. However, two-level interactions of X_1 and X_3 (X_{13}) and two-level interactions of X_2 and X_3 (X_{23}) terms are insignificant (with probability values larger than 0.05). Thus, these insignificant model terms (X_{13} and X_{23}) were eliminated and reanalysed and the results are shown in Table 5.

According to the ANOVA results depicted in Table 5, the developed reduced model was significant and well adapted to the response (ΔRGB) as shown by F-value = 4132.7, *p*-value less than 0.05 and the lack of fit > 0.05. Moreover, the R^2 value of 0.9996 was in good agreement with $R^2_{adjusted}$ value of 0.9993. The closeness of R^2 to $R^2_{adjusted}$ suggests a good relationship between experimental and predicted values. Thus, the developed reduced model was suitable for predicting the ΔRGB values in different independent variable combinations. The results revealed that concentration of cit-AuNPs (X_1), concentration of aptamer (X_2), incubation period (X_3), X_{11}, X_{22}, X_{33} and X_{12} were the significant factors influencing the response (ΔRGB) as indicated by their *p*-values less than 0.05. According to the results in Table 5, a second-order polynomial model was developed. Table 6 shows the regression results of the quadratic model for the ΔRGB at a 95% significance level.

Table 4. Analysis of variance (ANOVA) for ΔRGB from face centered central composite design (FCCCD) (*Full model*).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	5896.35	5896.35	655.15	4532.49	0.000
Linear	3	1725.9	1725.9	575.3	3980.06	0.000
X_1	1	1612.9	1612.9	1612.9	11158.43	0.000
X_2	1	28.9	28.9	28.9	199.94	0.000
X_3	1	84.1	84.1	84.1	581.82	0.000
Square	3	4161.45	4161.45	1387.15	9596.65	0.000
X_{11}	1	3920	1298.2	1298.2	8981.29	0.000
X_{22}	1	151.25	61.45	61.45	425.16	0.000
X_{33}	1	90.2	90.2	90.2	624.06	0.000
Interaction	3	9	9	3	20.75	0.000
X_{12}	1	8	8	8	55.35	0.000
X_{13}	1	0.5	0.5	0.5	3.46	0.093
X_{23}	1	0.5	0.5	0.5	3.46	0.093
Residual Error	10	1.45	1.45	0.14		
Lack-of-fit	5	0.61	0.61	0.12	0.73	0.628
Pure Error	5	0.83	0.83	0.17		
Total	19	5897.8				

R^2= 0.9998, $R^2_{(pred)}$ = 0.9990, $R^2_{(adj)}$ = 0.9995

Table 5. Analysis of variance (ANOVA) for ΔRGB from face centered central composite design (FCCCD) (*Reduced model*).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	7	5895.35	5895.35	842.19	4132.7	0.000
Linear	3	1725.9	1725.9	575.3	2823.03	0.000
X_1	1	1612.9	1612.9	1612.9	7914.6	0.000

X_2	1	28.9	28.9	28.9	141.81	0.000
X_3	1	84.1	84.1	84.1	412.68	0.000
Square	3	4161.45	4161.45	1387.15	6806.84	0.000
X_{11}	1	3920	1298.2	1298.2	6370.37	0.000
X_{22}	1	151.25	61.45	61.45	301.56	0.000
X_{33}	1	90.2	90.2	90.2	442.64	0.000
Interaction	1	8	8	8	39.26	0.000
X_{12}	1	8	8	8	39.26	0.000
Residual Error	12	2.45	2.45	0.2		
Lack-of-fit	7	1.61	1.61	0.23	1.38	0.373
Pure Error	5	0.83	0.83	0.17		
Total	19	5897.8				

$R^2 = 0.9996$, $R^2_{(pred)} = 0.9986$, $R^2_{(adj)} = 0.9993$

Table 6. Regression coefficients and constant for model development to predict ΔRGB.

Independent factor	Regression coefficient (coded values)
Constant	-294.740
X_1	2202.05
X_2	1.43580
X_3	0.285152
X_{11}	-3394.89
X_{22}	-0.295455
X_{33}	-0.00636364
X_{12}	3.12500

The second-order polynomial model is shown in Eq. 4 in terms of coded factors of the ΔRGB of MPA (Y):

$$Y = -294.740 + 2202.05 X_1 + 1.43580 X_2 + 0.285152 X_3 - 3394.89 X_{11} - 0.295455 X_{22} - 0.00636364 X_{33} + 3.12500 X_{12} \quad (4)$$

where Y is the response value (ΔRGB), X_1 is the concentration of cit-AuNPs (nM), X_2 is the concentration of DNA aptamer (mM) and X_3 is the incubation period (min). The best model to fit the experimental data with independent variables was the quadratic model. Based on Eq. 4, the ΔRGB have been 99.96 % that is affected by different factors. The main effects of X_1, X_2 and X_3 have the coefficients of +2202.05, +1.4358 and +0.285152, respectively. The coefficient of interaction effects of X_{12} is +3.125, and the highest square effects of the factors belong to the X_{11} with the coefficient of −3394.89 (Table 6).

4. Conclusion

A DNA aptamer-cit-AuNPs aptasensor for detection of MPA was successfully developed. The faced centred central composite design (FCCCD) of response surface methodology (RSM) found to be useful in optimizing DNA aptamer-cit-AuNPs conditions (concentration of cit-AuNPs, concentration of DNA aptamer and incubation period) for MPA detection. The statistical analysis showed that cit-AuNPs concentration, DNA aptamer concentration and incubation period, all square terms and interaction terms of cit-AuNPs concentration and DNA aptamer are the significant parameters influencing the ΔRGB. The developed reduced model for optimizing the variables is a second order polynomial model with R^2 value of 0.9996, demonstrating a good fitted model with the experimental results.

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