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Optimization of process parameters by Taguchi method for the development of biosensor for the detection of acrylamide in fried foods

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Abstract
In the present era, the consumer demand for food safety standards, especially in fried foods urge for a rapid detection of food toxins, specifically thermal process induced toxin such as acrylamide. Biosensor as a rapid detection technique is more preferred for acrylamide detection than other conventional instrumental techniques. Optimization of different process parameters for the development of biosensor is a necessity for its improved sensitivity. Design of experiment (DoE) using Taguchi model was utilised in this study, to optimize pH, Silver nanoparticles (AgNps) and haemoglobin (Hb) required for sensor fabrication with minimal experimental runs. Larger signal to noise ratio was applied in the Taguchi model to obtain the maximum current response. The optimal parameters obtained using Taguchi design was pH (4), AgNps (5 µl) and Hb (5 µl) with a significant difference (p<0.05). The developed model was validated by using linear regression model. Results obtained suggests that, the Taguchi method can be applied as an effective tool for the optimization parameters in development of biosensors for acrylamide detection.

Keywords: Acrylamide, biosensors, detection, optimization, DoE, Taguchi

Introduction

Acrylamide, a known human neurotoxin (Parzefall *et al.*, 2008; Tareke E *et al.*, 2002) [1, 2] and probable carcinogen (Tareke E *et al.*, 2002; Smith CJ *et al.*, 2001) [2, 3], enters the food chain mainly as a by-product of heat-processed carbohydrate-containing foods such as not only French fries, potato chips, crackers, but also coffee, black olives, and others (Parzefall *et al.*, 2008; Tareke E *et al.*, 2002; Dybing E *et al;* 2005) [1, 2, 4]. Fried foods, especially chips & crisps are one of the most convenient food options currently available for people to keep pace with their busy lifestyle. The prevalence of acrylamide in fried foods raised a public concern for the detection of acrylamide in fried food matrix. Some of the existing methods for detection of acrylamide are gas chromatography mass spectrometry (GC−MS), liquid chromatography mass spectrometry (LC-MS) and high-performance liquid chromatography mass spectrometry (HPLC−MS). Even though, the mentioned sophisticated analytical equipment is accurate, precise, and reliable, yet they are costly and time consuming. Hence, a quick detection method is needed which can be done by biosensors.

Biosensors analytical device, which integrates a living recognition entity viz. enzyme, antibodies, phages, aptamers or single, stranded DNA with suitable physicochemical, optical, thermometric, piezoelectric and magnetic transducers (Bahadir EB *et al.*, 2015) [5] In case of biosensors, the bio recognition element acts in a biochemical mechanism in combination to an optoelectronic system (Cammann *et al.*, 1977; Anthony *et al*; 1987) [6 7]. Electrochemical sensors measure the electric signals such as current/potential, which is directly proportional to the analyte concentration. Likewise, any other instrumental analysis Optimization of parameters plays a major role in the performance evaluation of the biosensor.

Nowadays, the optimization of various experimental process parameters was commonly done using different design of experiments (DoE) methods (Weissman *et al.*, 2015) [8]. The design of experiments (DoE) is a dominant multivariate statistical approach to acquire the highest potential amount of information about a system with lesser number of experimental runs. Lesser number of performing experimental runs on the basis of DoE will give a benefit of saving time, cost and materials used for experiments (Azadi A *et al.*, 2019; Ghasemi *et al.*, 2003) [9, 10].

There are many commercially available free soft wares such as Minitab, Origin Pro, SPSS, Prism etc are used to design and analyse the DoE. In addition to that, Microsoft Excel is a popular tool utilized for DoE by applying standard formulas and procedures (Granato *et al.*, 2014) [11]. Linear regression and analysis of variance (ANOVA) are necessary to understand in prior to conduct DoE for any optimization process. Furthermore, some of the basic necessary practical steps to execute a DoE includes: (a) Note down the objectives (b) defining the objectives (c) Choosing the dependant and independent variables alongside with levels and factors (d) Decide the experimental design type (e) Execution of experiment as per the designed matrix (f) Data analysis (e) Conclusion.

Some of the general approaches for DoE for optimization consists of Factorial designs with one, two, three and more factors, full factorial design and fractional factorial design. All of these mentioned approaches are quite complex with more number of experimental runs and in turn it increases the expense and time of the optimization process (Rao *et al.*, 2008; Durakovi *et al.*, 2012) [12, 13]. Taking in to consideration of all these complications, Dr. Genici Taguchi found a special factorial design called Taguchi model, which can considerably minimize the experimental runs and can be applied in various applications.

Taguchi method is a statistical design technique to improve various experimental factors presented by Taguchi and Konishi. Suitable selection of different parameters and splitting them in to control and noise factors to obtain the desirable results can be successfully achieved through this method. In Taguchi, the control parameters are performed to minimize the effect of noises. The results obtained in Taguchi experiments are utilized to scrutinize the data and, thereby anticipate the specificity of the designed method (Koorand *et al.*, 2018; Lazić *et al.*, 2013; Rao *et al.*, 2008; Khoei *et al.*, 2002) [14, 15, 12, 16]. The main advantages of Taguchi method include decreasing the number of experimental runs and thereby minimising the expenses involved in it, capacity to observe discrete factors, define the influence of various factors, capability to estimate the result data in optimal settings, determine error contribution and the opportunity to acquire optimal conditions for various responses at the same time (Koorand *et al.*, 2018) [14].

To the best of my knowledge there is no literature reported on application of Taguchi method for the optimization of parameters used in the development of biosensors for the detection of acrylamide in fried food matrix. This study was aimed to examine the effect of parameters such as pH, silver nanoparticles (AgNps) and haemoglobin (Hb) concentration for getting a better response in biosensor with minimized number of experimental runs.

2. Materials and Methods

2.1 Experimental materials and apparatus

Chemicals required for conducting experiments were bought from M/s Sigma Aldrich, India of Analytical grade: Acrylamide standard, Human haemoglobin (lyophilized powder), Phosphate buffer, Silver Nitrate, Trisodium Citrate, Chitosan (from shrimp shells, ≥75% (deacetylated) The screen printed electrode, with working electrode as glassy carbon, reference electrode as Ag/Ag Cl, and the counter electrode of platinum and the potentiostat Sensit BT:SNS configuration potentiostat (Palmsens 4) was brought from M/s Class One Systems, New Delhi.

2.2 Optimization of pH, AgNps and Haemoglobin concentration for development of biosensor using Taguchi Design of experiment (DoE)

Optimization of various process parameters are important in increasing the sensitivity of a biosensor. The selected parameters for optimization in this study involves pH of the phosphate buffer (PB), concentration of AgNps (µl) and 5 (mg/ml) of Hb in (µl). Experimental runs were conducted by dropping spiked acrylamide standard in PB buffer on the working electrode of screen printed glassy carbon electrode modified with AgNps and Hb. Then its response was noted. Taguchi orthogonal array method of L9 (3^3) using Minitab 17.0 software with the parameters pH, AgNps and Hb concentration are shown in Table 2. Analysis of variance (ANOVA) was performed for all the experimental runs to determine the significance of parameters for the response obtained from the fabricated biosensor. In each parameter level, signal to noise ratio (S/N ratio) was calculated in order to remove the noise factor caused during the optimization process. The present study requires maximum response, so larger S/N was applied, which was given by the following equation (Ghiasi *et al.*, 2021) [17].

$$S/N = -10 \log [1/n \, \Sigma \, (1/yi^2)] \ldots (1)$$

Where 'y' and 'n' are denoted as the observed data and number of observations, correspondingly.

2.3 Taguchi model analysis using linear regression model

A linear regression equation was obtained for the maximum current response recorded during the experiment. The current and S/N ratio obtained for the optimized parameters was applied in the obtained linear equation. In addition, the descriptive quality of the model was analysed using multiple correlation coefficient (R^2).

2.4 Validation and residual analysis for Taguchi model

The Taguchi model was validated the obtained experimental values for the optimized parameters. The residual analysis was carried out to understand whether the model fits the statistical assumptions and it was performed with a 95% level of confidence (Montgomery *et al.*, 2013) [18]. Relative error was calculated using the below equation.

$$\text{Relative error} = X = \frac{\text{Experimental value} - \text{Predicted value}}{\text{Predicted value}} \ldots (2)$$

3. Results and Discussions

3.1 Optimization of pH, silver nanoparticles and haemoglobin by Taguchi DoE

The optimized parameters obtained from Taguchi DoE was pH (4), AgNps (5µl), Hb (5 mg/ml). An orthogonal design of L9 (3^3) was performed using Minitab software according to Table 1.

From the figure 2 the optimized value of pH is 4 it may be because that the current obtained at this pH was more stable. Similar reports are reported by (Navarro *et al.*, 2020) [19]. The optimized value of silver nanoparticle (AgNps) found to be 5 (µl), the main reason was, it was observed that in this particular volume of AgNps, the current obtained from the biosensor follows a linear trend compare to other volumes. In the course of optimizing Hb concentration, it was found that 5(µl) Hb exhibit a higher linear current response. This may be because that the available Hb molecules on the working electrode surface might be free even after binding with the analyte acrylamide, which in turn reduces adduct formation hence it results in higher linear current. Similar results are reported by (Yadav *et al.*, 2017) [20].

Nonparallel lines in the interaction plot indicates there are interactions between the parameters. Cross lines indicate strong interactions (Kirati *et al.*, 2019) [21]. Figure 1 illustrates strong interactions between Hb and AgNps and pH and AgNps.

Table 3 shows the response table; it gives a relative degree comparison between the parameters by ranking them as per the delta values. The difference between the uppermost and the lowermost mean for each parameter was used to find the delta (Nia *et al.*, 2019) [22]. In this design, pH is the most significant parameter that determines the peak current followed by AgNps and Hb respectively where Hb is the least parameter which affects the peak current.

3.2 Analysis of variance (ANOVA)

In Taguchi design experiments, the ANOVA is generally used to understand the effect of parameters on the average response and S/N ratio. Table 4 illustrates the degree of freedom (DF), the sum of squares (SS), mean square (MS), F-value (F), and p-value on the basis of S/N data. The ANOVA determines the relative relevance of each parameter by means of the sequential (Seq) and adjusted (Adj) sum of squares, with the parameter with the highest SS having the major influence on the experimental settings. From the SS values, pH has the highest value followed by AgNps and Hb. In addition to that pH has the lowermost p-value (0.006) and Hb has the highest p-value (0.348). Lowest p-value i.e, $p \leq 0.05$ indicates pH and AgNps has significant effect in current response of the biosensor, whereas Hb with $p \geq 0.05$ indicates it has least effect in the current response. From this observations, it is clearly understood that even the slight variations in pH and AgNps can have major impact on the performance of the biosensor.

3.3 Linear regression model

The linear model equation was established for maximum current response. "Larger is the better" S/N was taken for current response using Equation 1. Equation 2 signifies the regression equation based on the results of S/N ratio.

Current (milliampere) = 261.1 - 24.55 pH -3.53 AgNps (microlires) - 4.36 Hb (micrlitres)… (1)

S/N ratio = 58.69 - 3.459 pH - 0.410 AgNps (microlires) - 0.229 Hb (micrlitres)… (2)

Multiple correlation coefficient (R^2) was applied to exemplify the model descriptive quality. The R^2 value of current and S/N ratio of models were 95.3% and 88.38% respectively. From the higher R^2 value, it can be assured that the experimental and predicted values are very close, and thus the regression model is very much significant.

3.3 Validation and residuals analysis of the Taguchi model

The model was validated with experimental values for the given parameters shown in Table 5. The relative error of current response and S/N ratio calculated for the given condition of parameters was 0.255 and -0.261 respectively. The negative sign of relative error in S/N ratio showed a fair agreement predicted model (Vyas *et al.*, 2019) [23].

Figure 3a represents the residual analysis applied to analyse if the model fits the statistical assumptions, i.e., that the residuals are random and normally distributed with a mean of zero and constant standard deviations, and that was assumed with a 95% level of confidence (Montgomery *et al.*, 2013) [18]. The normal probability plot shows that they are close to a straight line, denotes that the errors are normally distributed and the normality hypothesis is met (Figure 3 a). The variation of residuals as a function of fitted values is illustrated in Figure 3b. The standardized residuals generated a randomly distributed scattered point distribution within the range of ±40.

The random scattering of residuals round the surface shows that the model was appropriate, and the independence and constant variance assumptions were not overstepped. Therefore, the Taguchi model can be applied for the development of biosensor for acrylamide detection.

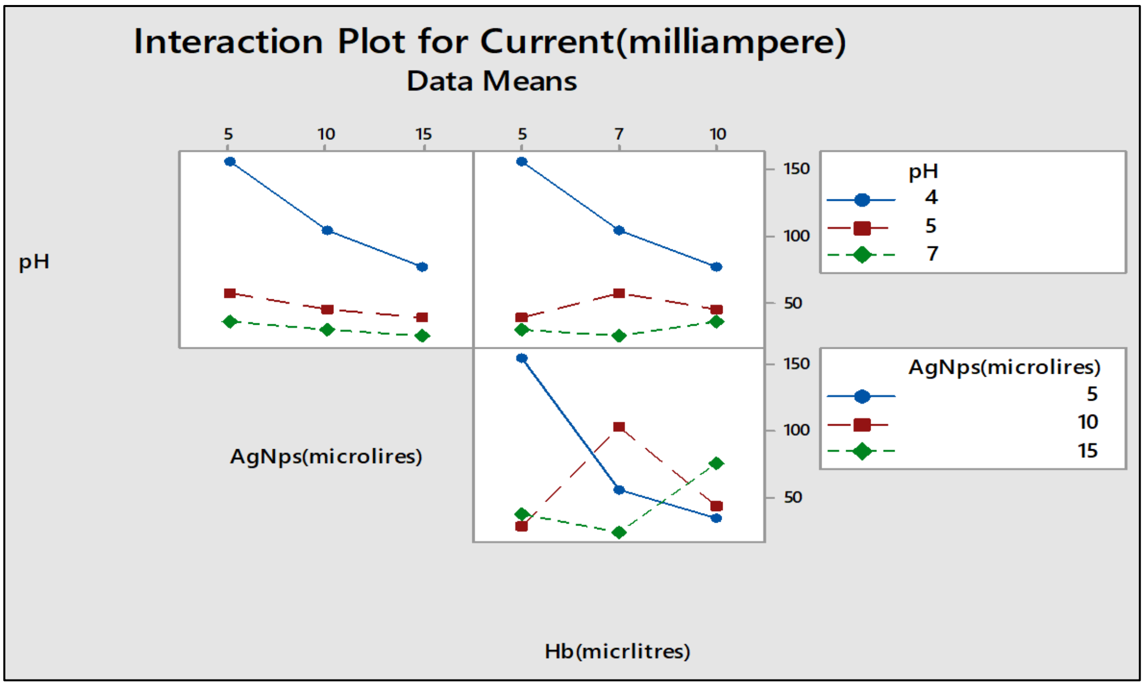

Fig 1: Interaction Plot for Current (milliampere)

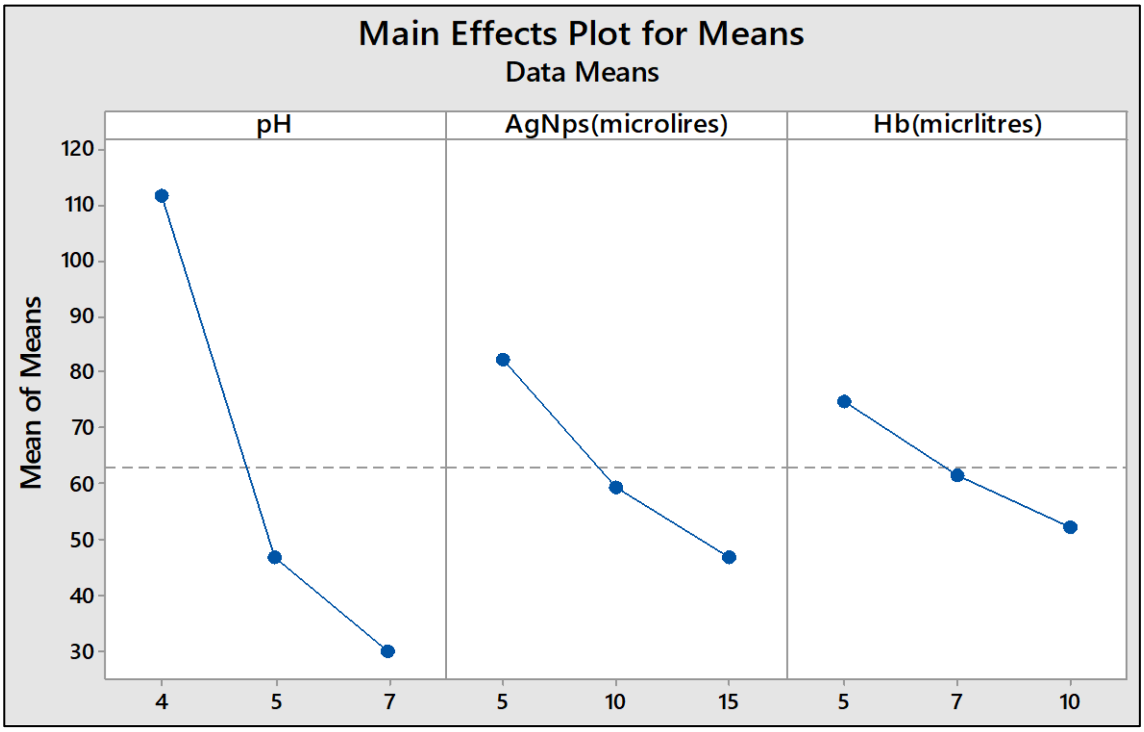

Fig 2: Main Effect Plot for Means

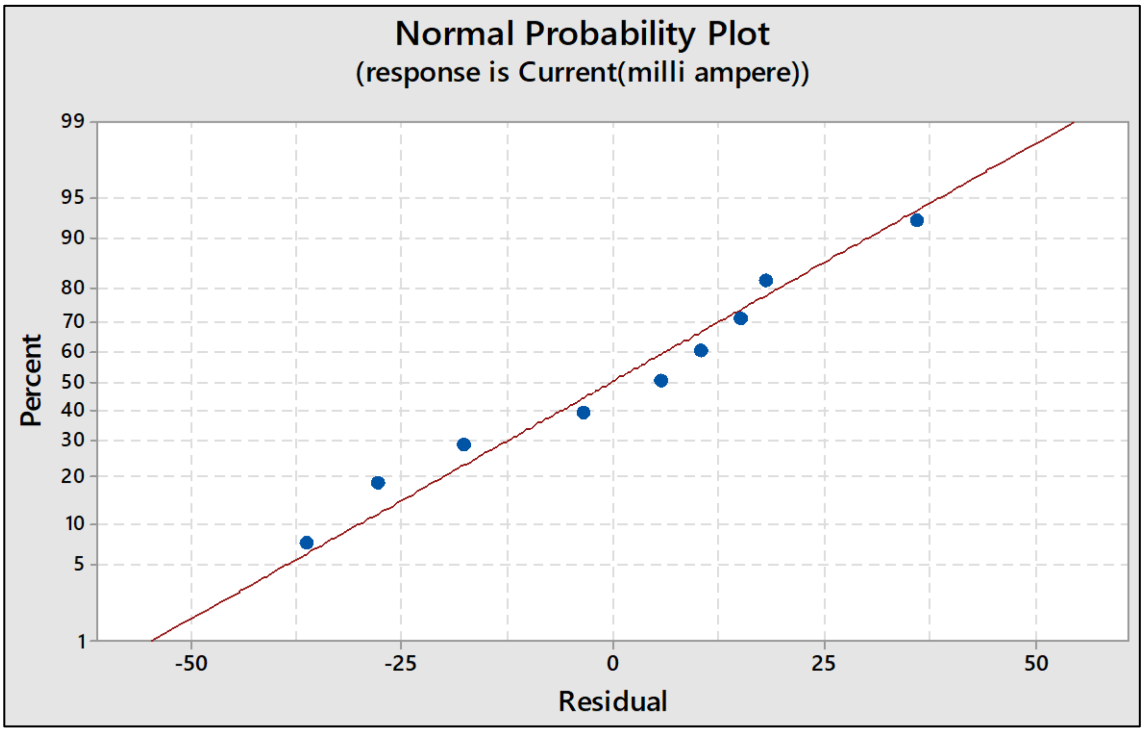

Fig 3a: Normal Probability Plot

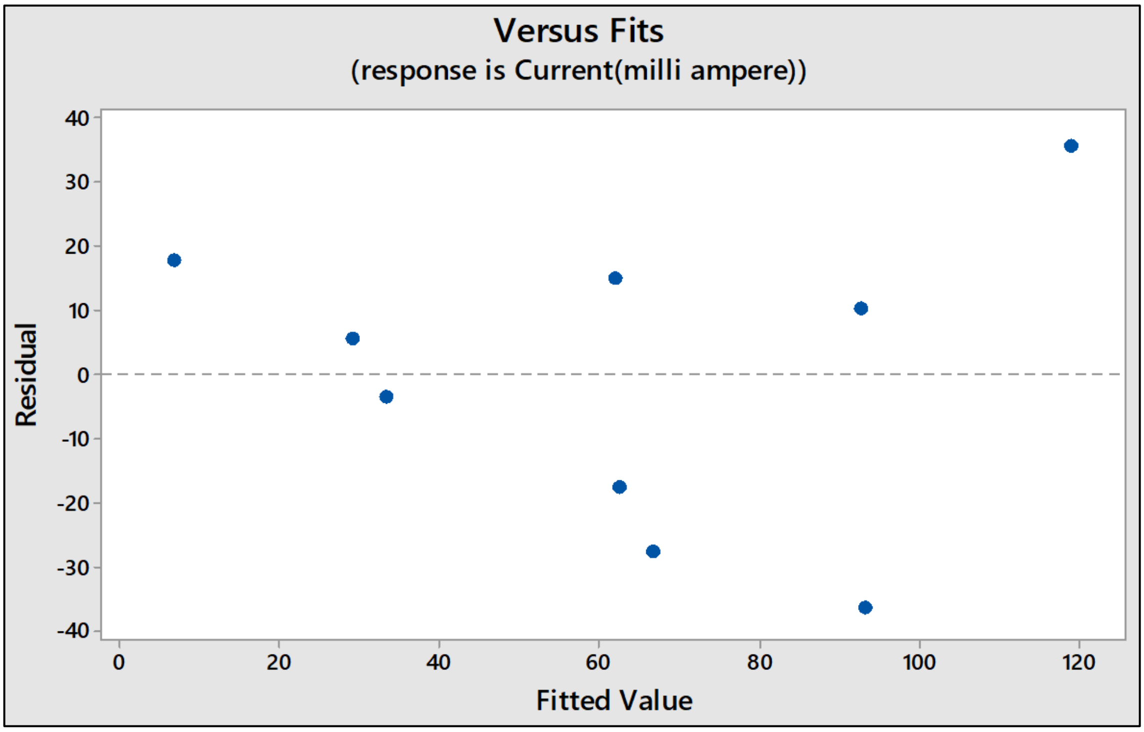

Fig 3 b: Versus Fits

Table 1: Process Parameters and Their Levels

Parameters	Levels		
pH	4	5	7
AgNps (µl)	5	10	15
Hb (mg/ml)	5	7	10

Table 2: Taguchi orthogonal array L9 (3^3) design of experiments

Runs	Variable		
	pH	AgNps(µl)	Hb (µl)
1	4	5	5
2	4	10	7
3	4	15	10
4	5	5	7
5	5	10	10
6	5	15	5
7	7	5	10
8	7	10	5
9	7	15	7

Table 3: Response Table for Signal to Noise Ratios Larger is better

Level	pH	AgNps(microliters)	Hb (µl)
1	40.60	36.60	35.06
2	33.33	34.29	34.44
3	29.46	32.50	33.89
Delta	11.14	4.10	1.16
Rank	1	2	3

Table 4: Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	191.790	191.790	95.8952	176.57	0.006
AgNps (microliters)	2	25.337	25.337	12.6685	23.33	0.041
Hb (microliters)	2	2.038	2.038	1.0188	1.88	0.348
Residual Error	2	1.086	1.086	0.5431		
Total	8	220.251				

Table 5: Validation of the model

	Parameters			Experimental	Predicted	Relative error
	pH	AgNps	Hb			
Current	4	5	5	155	123.45	.255
S/N	4	5	5	31.02	41.695	-0.261

Conclusion

The interactions study between the various parameters was necessary for biosensor development as per the literature, which includes pH, AgNps and Hb showed a good influence in the current response of the fabricated biosensor. In addition to that, an optimal condition for selected parameters for biosensor development was pH (4), AgNps (5 µl) and Hb (5 µl). The most significant and first ranking parameter which influence the biosensor activity was the pH. The R^2 value of linear response model and S/N ratio of models were 95.3% and 88.38% respectively, which is the evidence of the model's adaptability to the experimental conditions. The relative error between the current of the biosensor (experimental) and the predicted value for the given optimal conditions was minimal 0.255 and -0.261 respectively, which can be acceptable. The obtained results indicate that the Taguchi model DoE can be applied in development of biosensors with minimum number of experimental runs.

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