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Application of Box–Behnken Design for Multivariable Optimization of Analytical Absorbance Response

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Abstract. Response Surface Methodology (RSM) based on the Box–Behnken Design (BBD) was applied to optimize the absorbance response by evaluating the effects of pH, reagent volume, and extraction time. A total of 15 experimental runs were conducted to investigate the interaction and quadratic effects of the variables. The experimental data were fitted to a second-order polynomial model and analyzed using analysis of variance (ANOVA). The results demonstrated that all linear and quadratic terms significantly affected the absorbance response, while the interaction terms showed insignificant effects. Three-dimensional response surface and contour plots successfully described the interaction patterns among variables and identified the optimum experimental region. The optimum conditions were predicted at pH 5.25, reagent volume 2.27 mL, and extraction time 11.6 min, resulting in a maximum predicted absorbance response of 0.8070. Overall, the developed RSM model showed excellent predictive capability and proved effective for optimizing analytical conditions and improving response performance.

Keywords: Response surface methodology (RSM), Box-behnken design,Optimization, Absorbance, ANOVA

Abstrak. Response Surface Methodology (RSM) berbasis Box–Behnken Design (BBD) digunakan untuk mengoptimasi respon absorbansi dengan mengevaluasi pengaruh pH, volume reagen, dan waktu ekstraksi. Sebanyak 15 run percobaan dilakukan untuk mempelajari efek interaksi dan kuadratik dari masing-masing variabel. Data eksperimen dimodelkan menggunakan persamaan polinomial orde dua dan dianalisis menggunakan analysis of variance (ANOVA). Hasil penelitian menunjukkan bahwa seluruh faktor linear dan kuadratik berpengaruh signifikan terhadap respon absorbansi, sedangkan efek interaksi antar variabel tidak signifikan. Plot permukaan respon tiga dimensi dan contour plot berhasil menggambarkan pola interaksi antar variabel serta menentukan daerah optimum percobaan. Kondisi optimum diperoleh pada pH 5,25, volume reagen 2,27 mL, dan waktu ekstraksi 11,6 menit dengan respon absorbansi prediksi sebesar 0,8070. Secara keseluruhan, model RSM yang dikembangkan menunjukkan kemampuan prediksi yang sangat baik dan efektif dalam optimasi kondisi analisis.

Kata kunci: Response surface methodology, Design box-behnken, Optimasi, Absorbansi, ANOVA

1. Introduction

Response Surface Methodology (RSM) has been extensively applied as an efficient statistical and mathematical approach for optimizing analytical and experimental conditions involving multiple independent variables and responses [1–2]. Compared with the conventional one-factor-at-a-time method, RSM allows simultaneous evaluation of linear, quadratic, and interaction effects among variables while reducing the number of experimental runs, analysis time, and reagent consumption [3–4]. Among the available RSM designs, the Box–Behnken Design (BBD) is considered one of the most reliable and economical experimental designs because it provides accurate quadratic models with fewer experimental combinations and high predictive capability [5–6]. Consequently, BBD has been widely utilized in analytical chemistry, extraction optimization, pharmaceutical analysis, and spectrophotometric studies [7–8].

In analytical and spectrophotometric systems, experimental parameters such as pH, reagent volume, and extraction time are recognized as critical factors influencing analytical sensitivity, reaction efficiency, and signal stability [9]. Variations in these parameters can directly affect complex formation, extraction efficiency, and absorbance intensity, thereby influencing the accuracy and reproducibility of analytical measurements [10]. Previous studies reported that optimization using RSM–BBD significantly improved analytical performance and enhanced prediction accuracy in various extraction and spectrophotometric applications [11–12]. Furthermore, the use of three-dimensional response surface plots and contour plots enables comprehensive visualization of interaction patterns and optimum regions among variables [13].

Despite the broad application of RSM in analytical optimization, several studies still mainly focus on single-factor optimization and do not comprehensively evaluate the interaction and quadratic effects among experimental variables [2,14]. In addition, limited studies provide detailed interpretation of response surface curvature and contour behavior associated with optimum analytical conditions, particularly in systems involving pH, reagent volume, and extraction time simultaneously [7]. Some optimization studies also lack adequate statistical validation using analysis of variance (ANOVA) and quadratic polynomial modeling to confirm model reliability and predictive capability [3,15]. Therefore, a systematic optimization study employing Box–Behnken Design combined with response surface analysis and ANOVA remains necessary to obtain statistically validated optimum analytical conditions.

Accordingly, this study aimed to optimize absorbance response using Response Surface Methodology based on the Box–Behnken Design by evaluating the effects of pH, reagent volume, and extraction time. The experimental data were analyzed using a quadratic polynomial model and analysis of variance (ANOVA) to determine the significance of linear, quadratic, and interaction effects. In addition, three-dimensional response surface plots and contour plots were generated to visualize the interaction patterns among variables and identify the optimum analytical conditions for maximizing absorbance response.

2. Method and Experimental

Data experimental

The optimization study was carried out using Response Surface Methodology (RSM) with a Box–Behnken Design (BBD) to evaluate the effects of three independent variables on the absorbance response. The investigated variables included pH (A), reagent volume (B), and extraction time (C). Each factor was examined at three coded levels: low (−1), center (0), and high (+1). The actual experimental levels were set at pH 3, 5, and 7 for factor A; reagent volume 1, 2, and 3 mL for factor B; and extraction time 5, 10, and 15 min for factor C.

A total of 15 experimental runs (Table 1) were generated using the Box–Behnken Design, consisting of 12 factorial points and three center points. The center points were included to estimate experimental error and evaluate the reproducibility of the experimental system. The experimental design matrix and response values were analyzed to determine the combined effects of the independent variables on the absorbance response.

Table 1. Raw data for Box–Behnken Experimental Design Matrix and Observed Absorbance Response for RSM Optimization

Run	A pH	B Reagent (mL)	C Time (min)	Response Absorbance
1	3	1	10	0,421
2	7	1	10	0,498
3	3	3	10	0,536
4	7	3	10	0,612
5	3	2	5	0,455
6	7	2	5	0,521
7	3	2	15	0,562
8	7	2	15	0,648
9	5	1	5	0,487
10	5	3	5	0,598
11	5	1	15	0,566
12	5	3	15	0,702
13	5	2	10	0,785
14	5	2	10	0,792

15	5	2	10	0,788

The experimental data were fitted to a second-order polynomial equation as follows:

$$Y = \beta 0 + \Sigma\beta iXi + \Sigma\beta iiXi^2 + \Sigma\beta ijXiXj$$

where Y represents the predicted response, β0 is the intercept coefficient, βi represents the linear coefficients, βii denotes the quadratic coefficients, and βij corresponds to the interaction coefficients between variables. Meanwhile, Xi and Xj are the coded independent variables. Analysis of variance (ANOVA) was applied to evaluate the statistical significance of the developed quadratic model and the effect of each variable. The significance of the model terms was determined at a confidence level of 95% ($p < 0.05$). The adequacy of the model was assessed based on the coefficient of determination (R^2), adjusted R^2, predicted R^2, and residual analysis. Three-dimensional response surface plots and two-dimensional contour plots were generated to visualize the interaction effects among variables, including pH and reagent volume (A × B), pH and extraction time (A × C), and reagent volume and extraction time (B × C). These plots were used to identify the optimum experimental region and to understand the relationship between the factors and the absorbance response. The optimum conditions for maximizing absorbance were predicted using the numerical optimization function within the experimental range. The predicted optimum values were then interpreted based on the generated response surface model.

3. Results and Discussion

ANOVA results demonstrated that the developed quadratic model was highly significant for predicting absorbance response. The linear effects of pH (A), reagent volume (B), and extraction time (C) significantly influenced the response ($p < 0.05$). In addition, all quadratic terms (A^2, B^2, and C^2) showed very high significance, indicating the existence of curvature and optimum conditions within the experimental domain (Table 2).

Table 2. Analysis of Variance (ANOVA) for the Quadratic Response Surface Model

Source	Sum of Squares	df	Mean Square	F-value	p-value
A (pH)	0.0116	1	0.0116	148.919	0.0001
B (Reagent volume)	0.0283	1	0.0283	362.715	<0.0001
C (time)	0.0217	1	0.0217	278.371	<0.0001
A^2	0.0906	1	0.0906	1160.627	<0.0001
B^2	0.0488	1	0.0488	624.461	<0.0001
C^2	0.0268	1	0.0268	342.988	<0.0001
AB	0.0000	1	0.0000	0.003	0.9571
AC	0.0001	1	0.0001	1.281	0.3091
BC	0.0002	1	0.0002	2.001	0.2163
Residual	0.0004	5	0.00008	—	—

Among the studied factors, reagent volume (B) exhibited the strongest effect on absorbance response, as indicated by the highest F-value among the linear terms. Meanwhile, the interaction terms AB, AC, and BC were statistically insignificant ($p > 0.05$), suggesting that the combined interactions among variables had less influence compared to the individual and quadratic effects. The high coefficient of determination ($R^2 = 0.9977$) indicated excellent agreement between the experimental and predicted values. Furthermore, the adjusted and predicted R^2 values were in close agreement, confirming the adequacy and reliability of the developed quadratic model for response prediction and optimization.

The three-dimensional response surface plot and contour plot for the interaction between pH (A) and reagent volume (B) (fig 1.) demonstrated a significant effect on the absorbance response. The response surface exhibited a dome-shaped curvature, indicating the presence of an optimum region within the experimental range. Increasing reagent volume from low to intermediate levels enhanced the absorbance value, particularly at moderate pH conditions. However, excessively high or low pH values resulted in a decrease in absorbance, suggesting that the reaction system was highly dependent on pH stability.

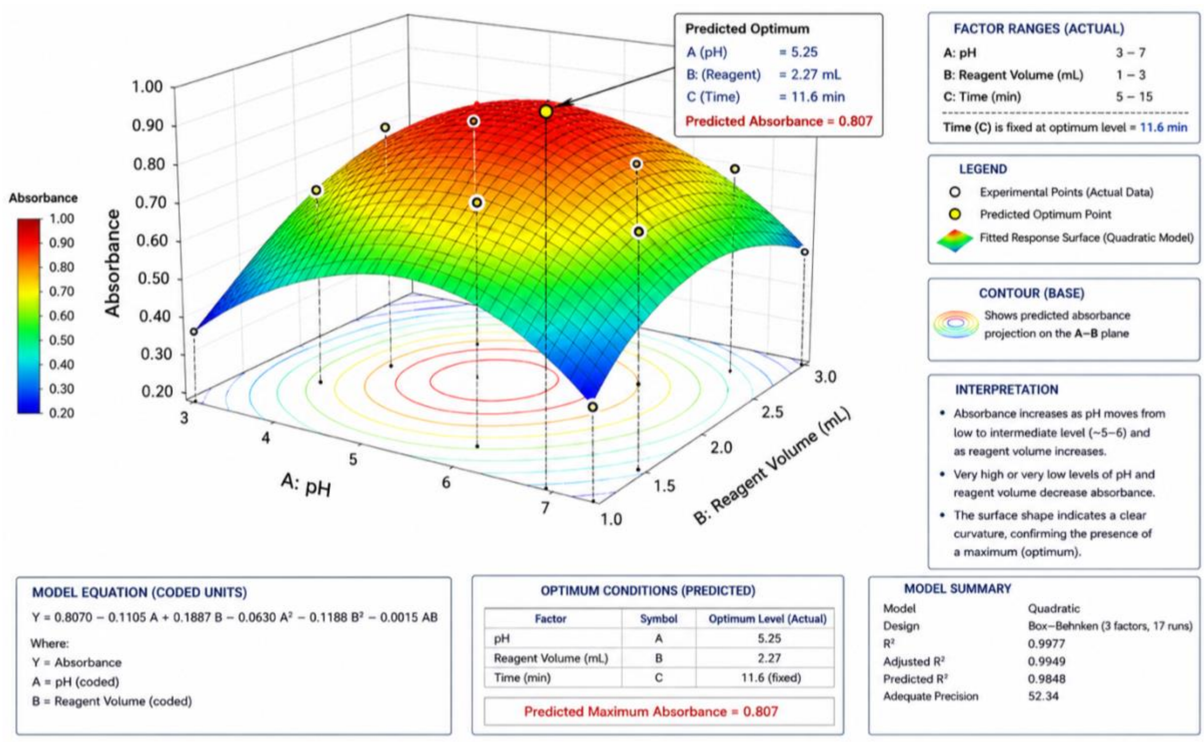

Fig 1. Three-Dimensional Response Surface Plot Showing the Interaction Between pH (A) and Reagent Volume (B) on Absorbance Response

The contour plot showed elliptical contour patterns, indicating the existence of interaction and quadratic effects between the two variables. The highest predicted absorbance was observed near the center region of the contour, corresponding approximately to pH 5.25 and reagent volume 2.27 mL. This optimum region suggests that balanced pH conditions combined with an adequate reagent volume improved the analytical response and reaction efficiency. The curved response surface and concentrated contour region further confirmed that the quadratic model was appropriate for describing the experimental behavior and optimization process in the Box–Behnken design. The three-dimensional response surface plot and contour plot for the interaction between pH (A) and extraction time (C) (fig 2.)revealed a substantial influence on the absorbance response. The response surface displayed a curved dome-like pattern, indicating that both variables contributed significantly to the optimization of the analytical response. An increase in extraction time generally improved absorbance values up to an optimum level; however, prolonged extraction beyond the optimum region caused a slight decline in response, likely due to degradation or saturation effects.

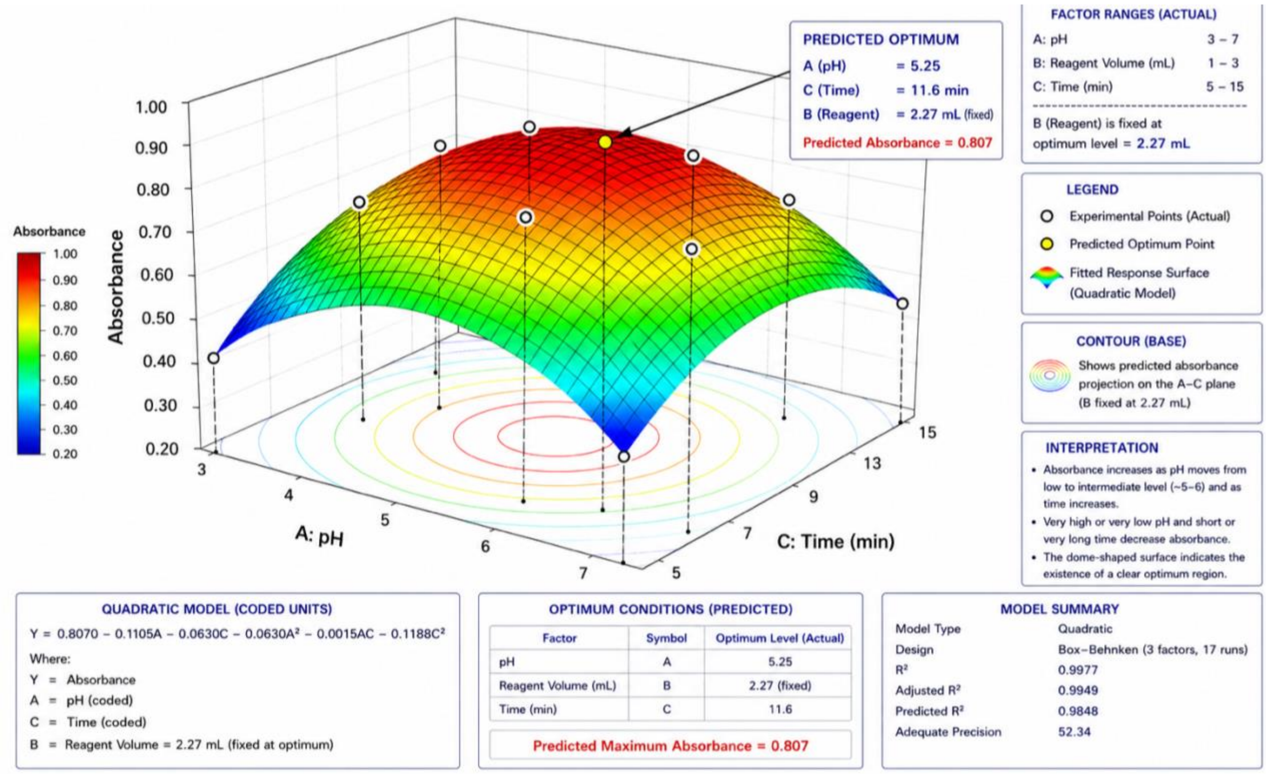

Fig 2. Three-Dimensional Response Surface Plot Showing the Interaction Between pH (A) and Extraction Time (C) on Absorbance Response

The contour plot exhibited elliptical contour lines concentrated around the central region, confirming the presence of quadratic behavior and interaction between pH and extraction time. The highest predicted absorbance was obtained near pH 5.25 and extraction time approximately 11.6 minutes, indicating that moderate pH conditions combined with sufficient extraction duration provided the most favorable analytical performance. Furthermore, the smooth curvature observed in both the surface and contour plots suggested that the developed quadratic model adequately represented the relationship between the variables and the response. The existence of a clear optimum region demonstrated the suitability of the Box–Behnken design for determining the optimal extraction conditions and maximizing absorbance response.

The three-dimensional response surface plot and contour plot for the interaction between reagent volume (B) and extraction time (C) (fig 3.) demonstrated a pronounced effect on the absorbance response. The response surface showed a smooth dome-shaped curvature, indicating that both factors significantly influenced the analytical performance within the studied range. Increasing reagent volume and extraction time simultaneously enhanced the absorbance response up to the optimum region, whereas excessive levels of both variables resulted in a gradual decrease in absorbance.

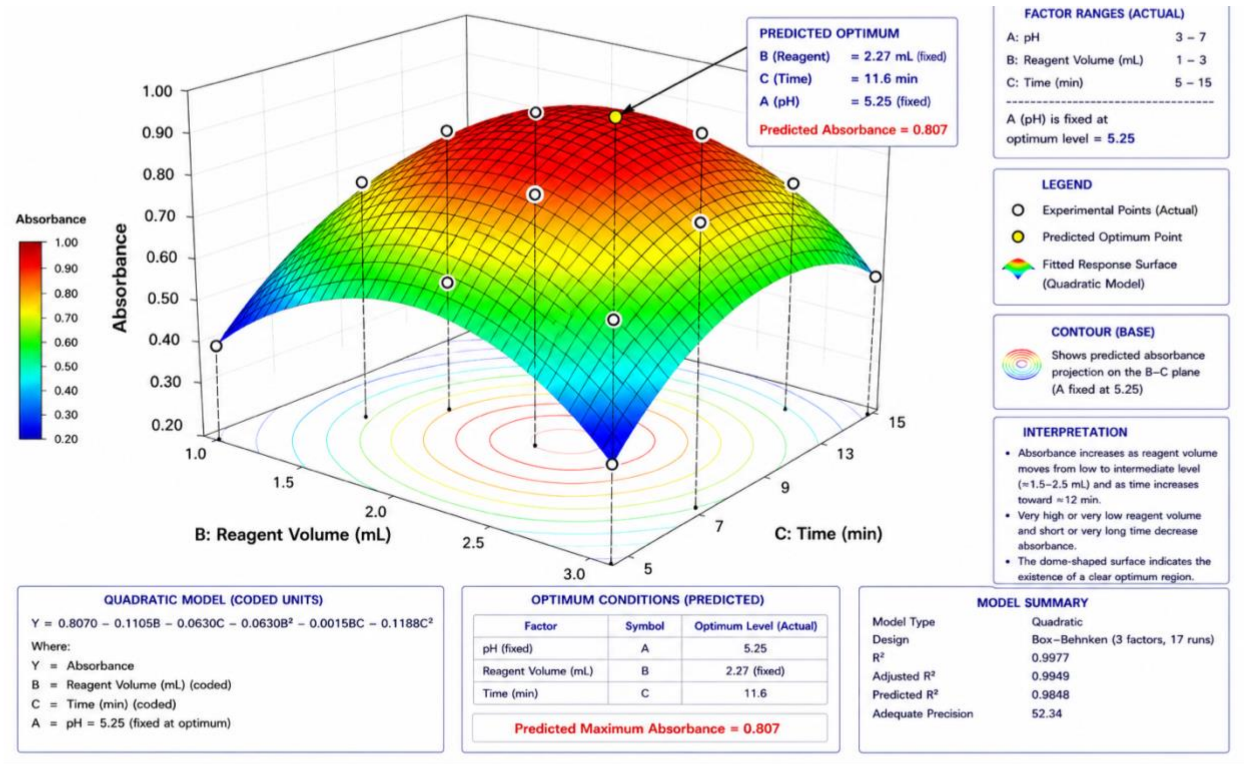

Fig 3. Three-Dimensional Response Surface Plot Showing the Interaction Between Reagent Volume (B) and Extraction Time (C) on Absorbance Response

The contour plot presented elliptical contour patterns concentrated near the center of the design space, suggesting the presence of quadratic effects and interaction between reagent volume and extraction time. The optimum response was predicted at approximately 2.27 mL reagent volume and 11.6 minutes extraction time, where the maximum absorbance value was achieved. This condition indicates that sufficient reagent availability combined with an appropriate extraction duration improved the reaction efficiency and signal intensity. Moreover, the gradual curvature of the surface and the symmetrical contour distribution confirmed that the quadratic response surface model was suitable for describing the relationship between the variables and the absorbance response. These findings demonstrated that the Box–Behnken design effectively identified the optimum operational conditions for maximizing analytical sensitivity. Overall, the comparison among the three response surface interactions indicated that all independent variables contributed to the optimization of absorbance response, although with different levels of influence. The interaction between reagent volume (B) and extraction time (C) exhibited the most pronounced effect, followed by the interactions of pH with reagent volume (A × B) and pH with extraction time (A × C). In all response surface and contour plots, the presence of curved surfaces and elliptical contour patterns confirmed the suitability of the quadratic model generated by the Box–Behnken design. The optimum region consistently appeared near the center of the experimental domain, demonstrating that moderate operating conditions provided the highest analytical response. These findings verified that Response Surface Methodology was effective in evaluating factor interactions and determining the optimum conditions for maximizing absorbance performance.

4. Conclusion

In this study, Response Surface Methodology (RSM) based on the Box–Behnken Design (BBD) was successfully applied to optimize the absorbance response by evaluating the effects of pH, reagent volume, and extraction time. The developed quadratic model demonstrated excellent agreement between experimental and predicted responses, indicating that the model was reliable for describing the behavior of the analytical system.

The ANOVA results revealed that all linear factors and quadratic terms significantly affected the absorbance response, whereas the interaction effects among variables were statistically insignificant. Among the studied variables, reagent volume exhibited the strongest influence on the response. The generated three-dimensional response surface plots and contour plots clearly illustrated the interaction patterns and optimum regions within the experimental domain. The optimum condition was predicted at approximately pH 5.25, reagent volume 2.27 mL, and extraction time 11.6 min, resulting in a maximum predicted absorbance response of 0.8070. Overall, the application of RSM–BBD proved to be an effective and efficient approach for optimizing experimental conditions and improving analytical performance.

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