

Optimization Central Composite Design for the Simultaneous Determination of Copper in Fruit and Vegetable Samples with Adsorptive Stripping Voltammetry in the Presence of Calcein

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Abstract

The purpose of this study was to optimize the simultaneous determination of trace copper by adsorptive stripping voltammetry in the presence calcein as a complexing agent. The independent factors that affect the response were calcein concentration, pH, potential accumulation and time accumulation. The research design was used a central composite design (CCD) with 4 variables, 5 level and 31 a combination of treatments. The first step of 2^k factorial design optimization is: to give the highest level of code values (+1.68), the lowest level (-1.68) and code (0) as the center point. Programs that will be used for statistical data processing, namely Minitab 17 using CCD. Based on data analysis with response surface method, the obtained optimum conditions for the simultaneous determination of copper is calcein concentration 0.11 mM, pH 6.8, potential accumulation -0.88 V and time accumulation 42.34 s. The linear calibration graph for the concentration (0.2 -110.0) $\mu\text{g/L}$, with detection limits 1.79 $\mu\text{g/L}$. This procedure was successfully applied for the determination of in the fruit and vegetable samples with satisfactory results.

Keywords: Simultaneous determination; Copper; Adsorptive stripping voltammetry; Central composite design; Fruit and vegetable samples

Introduction

Some of the elements included in the category of heavy metals such as As, Cr, Cd, Pb, Fe, Cu, Co, Hg, Se, Sb, Mn, Zn and Ni come from industrial waste and the results of the activity of the population. Copper is essential for living organisms at only trace amounts, but when it exceeds the prescribed limit, it has also a detrimental effect on human health. Exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity and developmental toxicity [1]. Therefore, due to applications of this element in chemical industry it is very

important to be able to determine of trace copper in fruit and vegetable samples with highly sensitivity and selectivity. If it enters into the human body has a tendency to congregate in organs. could not get out again through the digestive process. Water, land and air are the medium that can be used for the deployment of heavy metals into the environment. Broad-leafed plants, in addition, to absorb heavy metals from the soil can absorb heavy metals from the air.

The previous studies. it has been reported by various researchers in the world, some determination of Cu and Cd acid dithiosalisilate 2.2 [2], the simultaneous determination of Pb and Cd with morin [3], the simultaneous determination of Cu and Cd ultra-trace levels in food samples and water types complexing agent were used for the analysis of the determination of metal ions either individually or simultaneously with adsorptive stripping voltammetry. Simultaneous with luminol [4], the simultaneous determination of Cu, Pb, Cd and Zn using murexide as a chelating agent [5], the simultaneous determination of Cd, Cu and Pb with calcon [6], the simultaneous determination of Pb and Cd at the level of a trace in natural waters with pyrogallol red [7], the simultaneous determination of Cu, Pb, Cd, Ni, Co and Zn in samples of bioethanol fuel with a mixture of several as a complexing agent by AdSV and regression multi-variant [8] and the simultaneous determination of Pb, Cd and Zn using clioquinol as a complexing [9], the simultaneous determination of Cd, Cu, Pb and Zn using calcein [10] and the simultaneous determination of Cu, Pb and Cd [11]. The determination is very sensitive to a number of trace Cu in food samples with 1.10 fenantrolin by adsorptive stripping voltammetry [12] and the determination of Cu in the ultra-trace levels in food samples with thiosemicarbazide [4].

AdSV was selected as an alternative method because it has many advantages such as high salinity of the sea water does not interfere with the analysis, high sensitivity, detection limit low on the scale ug/L (ppb), simple sample preparation, rapid analysis, low infrastructure cost [13-20]. In addition. with this method, it is possible to learn chemical species of heavy metals [21,22], which cannot be done with other methods. From the results of previous research, to find the optimization the determination of metal ions Cd, Cu, Pb and Zn [6,14] and metal ions Fe, Co, Ni and Cr¹⁷ either in the form of single or simultaneous AdSV, carried out by observing the effect of one variable is changed, while the other variables are kept at a constant level. This optimization technique is called optimization of the variables or the factors at the current time.

The main drawback of the results of the optimization of the factors that such optimization does not take into account the effect of the interaction between the variables studied. Therefore. this technique does not describe the full effect on the response parameter [23]. Another disadvantage of the optimization of the factors is the increased number of trials required to do research. which leads to increased time and increased consumption of reagents and materials. To overcome this problem. a technique of optimization of analytical procedures is by using multivariate statistical techniques. Calcein was selected as a sensitive and selective as a complexing agent has been reported by Marcin and Golimowski [24] on the determination of Ti. but calcein used in the determination of Cu with optimization central composite design has not been reported.

AdSV is a highly sensitive technique, the response obtained in the form of the peak current (Ip) was influenced by variables the following, namely: the effect variations of calcein concentration, pH, potential accumulation and, the time accumulation [5-6,11,13-18]. Therefore, it is very important to determine the optimization of these parameters, which will affect the measurement of the peak current in order to improve the quality of analytical results [25]. The research design was used in this study. as a tool for optimization was: CCD with 4 is coding, where the value of the highest level (+1.68). the lowest level

(-1.68) and code (0) as the center point. variables. 5 level/degree and 19 a combination of treatments. The first step of 2^k factorial design optimization Programs for statistical data processing Minitab 17 by using RSM (Response Surface Methodology).

The use of RSM has been reported by Yilmaz et al. [26] in the determination of Al content in salt samples using calcon as a complexing agent in AdSV. Furthermore Paolicchi et al. [25] have used the experimental design in the form of CCD optimization for determination of In (III) in the AdSV using pyrrolidine ammonium dithiocarbamates (APDC) as a complexing agent [26]. Danielle et al. [8] have reported the simultaneous determination of Cu, Pb, Cd, Ni, Co and Zn in the AdSV using multivariant regression aided optimization techniques with projection algorithm (SPA-MLR). Espada-Bellido [27] have used multivariant analysis for optimization determination of lead in the water AdSV at nanomolar levels based on the formation of metal complexes with 2-asetilpiridin salicyl hydrazone (2-APS). Deswati et al. [28] has been using CCD for the determination of cadmium in seawater using calcon by adsorptive stripping voltammetry and optimization determination and Zn in water samples. Furthermore, Deswati et al. [29] have reported CCD for optimization of a lead with calcon as a complexing agent by adsorptive stripping voltammetry.

The purpose of this study was to obtain the optimum condition of the heavy metal ion Cu. so can be applied to the analysis of these metals in samples of fruits. To achieve these objectives. required an optimization technique that was using the analytical procedure RSM with CCD [28-32]. To determine the accuracy, precision and sensitivity of the method are determined relative standard deviation (SDR), recovery and the limit of detection and the linearity of the proposed method.

Experimental Details

Equipment and materials research

The tools were used in this study were 797 Metrohm Computrace with HMDE working electrode, reference electrode such as Ag/AgCl/KCl, a Pt electrode as a supporting electrode, pH meter 80 models Griffin. Griffin and George Loughborough, England and analytical balance Mettler AE 200, Toledo OH-USA and glassware used in the laboratory.

Work procedures

Voltammeter into the vessel put 10 ml of standard solution of Cu (II) 10 $\mu\text{g/L}$. added 0.2 mL and 0.2 mL of 0.1 M KCl calcein in 20 ml. this variable is set constant during the experiment. The concentration of calcein. pH. accumulation potential and accumulation time are set according to the experimental design of the CCD in TABLE 1 below. From TABLE 1 is obtained peak current response in the form of metal ions Cu.

TABLE 1. Design Experiments RSM for simeultaneous determination of trace copper.

Exp	Factor				Respon (Ip)
	Calcein concentration (mM)	pH	Potential (V)	Time (s)	
1	0.1	6	-1.0	30	288.67
2	0.1	6	-0.8	30	289.30
3	0.1	6	-1.0	70	304.30
4	0.1	6	-0.8	70	318.07
5	0.1	8	-1.0	30	340.83

6	0.1	8	-0.8	30	349.40
7	0.1	8	-1.0	70	155.48
8	0.1	8	-0.8	70	152.32
9	0.3	6	-1.0	30	105.23
10	0.3	6	-0.8	30	120.92
11	0.3	6	-1.0	70	147.02
12	0.3	6	-0.8	70	152.42
13	0.3	8	-1.0	30	235.45
14	0.3	8	-0.8	30	228.63
15	0.3	8	-1.0	70	371.66
16	0.3	8	-0.8	70	256.74
17	0.2	7	-0.9	50	365.33
18	0.2	7	-0.9	50	365.40
19	0.2	7	-0.9	50	366.05
20	0.2	7	-0.9	50	366.30
21	0.2	7	-0.9	50	365.20
22	0.2	7	-0.9	50	361.74
23	0.2	7	-0.9	50	361.45
24	0.04	7	-0.9	50	289.45
25	0.2	5.4	-0.9	50	227.51
26	0.2	7	-1.06	50	245.17
27	0.2	7	-0.9	18.1	212.28
28	0.36	7	-0.9	50	243.76
29	0.2	8.6	-0.9	50	300.23
30	0.2	7	-0.74	50	312.72
31	0.2	7	-0.9	81.9	311.31

Design of research

The design of the study was: CCD with RSM 4 factors and 5 levels of each factor is coded -1.68, 0 and +1.68 with 2 replications. Score -1.68, 0. And +1.68 is a symbol that indicates the value of the variable. Score -1.68 indicates the value of the lowest variable +1.68 figures show the highest variable values. and the number 0 indicates the value of the variable medium. Factors of the research design were the concentration of calcein. pH. accumulation potential and accumulation time. The statistical data processing program for the Minitab 17 using RSM.

Determination of sampling site and sample preparation

The sampling method is done by purposive sampling, which is done by taking into account the condition of the research area also the current and the depth of the research area. This is intended to see to what extent the concentration of pollutants spread. To know the content of heavy metals in sea water is done sampling in sea water taken around Bungus, water tap from research laboratory of Chemistry Department, lake water from Maninjau Lake and river water taken from Lubuk Minturun. Samples of water prior to analysis were preserved with 65% HNO₃ with a ratio of 2: 1000 and filtered using Whatman 42 filter paper [30].

Vegetable samples used in this study are, cauliflower, mustard greens, eggplant and broccoli, while the fruits used include apples, banana kepok, melon and dragon fruit. Samples of vegetables and fruits before the determination of Zn metal content is determined first the water content, then do wet destruction with the addition of nitric acid pa and perchloric acid pa.

Each sample of 0.5000 g was carefully weighed and mashed, put into a Kestergum digestion flask. Further, the wet was destructed by the addition of 5 ml of concentrated nitric acid and 0.5 ml of concentrated perchloric acid, shaken and left overnight. Next, heated to the digestor block starting at 100°C, after the yellow vapor is exhausted the temperature is increased to 200°C. The destruction is terminated when the white vapor and liquid in the flask remain about 0.5 mL, cooled and diluted with doubly distilled water and the volume is adjusted to 50 mL, shaken until homogeneous and left overnight and disarrayed [31,32].

Clear solution is ready to be determined by Zn metal content with Voltammeter and Atomic Absorption Spectrophotometer as a comparison. The determination of the optimum conditions used in this study is the procedure developed by the previous research team [4, 7-9,11,12,17,18].

Detection method

The procedure of determining RSD (relative standard deviation), LOD (limit of detection), LR (linear range) and recovery has been done by previous researchers. The procedure of determining RSD (relative standard deviation), LOD (limit of detection), LR (linear range) and recovery has been done by previous researchers [4,7-9,11,12,17,18].

Results, Discussion and Conclusion

Optimization of copper using one variable

The optimization by observing the effect of one variable in the determination of trace metal Cu in the presence of calcein has been done [1]. The optimum condition as follow: calcon concentration 0.7 mM, pH 7, accumulation potential -0.6 V, accumulation time 50s. At the optimum conditions, the relative standard deviation was obtained with 8 replicates (n=8) measurements standard solution Cu (II) 10 µg/L was 0.86%, respectively. This technique does not describe the full effect on the response parameters and optimization of these factors is the increase in the number of trials required to do research, which leads to increased time and increased consumption of reagents and materials. Therefore to overcome this problem, an optimization technique of analytical procedures is by using CCD.

Optimization CCD for simultaneous determination of copper

The first order model can not be used. then proceed with the second order model by adding a quadratic effect and interaction. The result of data processing obtained the following:

TABLE 2. The regression coefficient on the second order model.

Term	Co-efficient
Constant	362.610
X ₁	-30.342
X ₂	22.480
X ₃	1.513
X ₄	3.054
X ₁ *X ₁	-31.944
X ₂ *X ₂	-32.908
X ₃ *X ₃	-27.581
X ₄ *X ₄	-33.643
X ₁ *X ₂	48.076

$X_1 * X_3$	-7.529
$X_1 * X_4$	35.977
$X_2 * X_3$	-9.489
$X_2 * X_4$	-20.987
$X_3 * X_4$	-7.311

According on TABLE 2. the model obtained from the data analysis were:

$$\hat{y} = 362.610 - 30.342 x_1 + 22.480 x_2 + 1.513 x_3 + 3.054 x_4 - 31.944 x_1^2 - 32.908 x_2^2 - 27.581 x_3^2 - 33.643 x_4^2 + 48.076 x_1 x_2 - 7.529 x_1 x_3 + 35.977 x_1 x_4 - 9.489 x_2 x_3 - 20.987 x_2 x_4 - 7.311 x_3 x_4$$

whereas the results of analysis of variance experimental data can be seen in TABLE 3.

TABLE 3. Results of analysis of variance the second order model.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	176846	176846	12631.8	6.62	0.000
Linier	4	31136	31136	7783.9	4.08	0.018
X_1	1	19939	19939	19938.9	10.44	0.005 (S)
X_2	1	10945	10945	10945.0	5.73	0.029 (S)
X_3	1	50	50	49.6	0.03	0.874
X_4	1	202	202	202.0	0.11	0.749
Square	4	77769	77769	19442.3	10.18	0.000
$X_1 * X_1$	1	22839	17096	17096.3	8.95	0.009
$X_2 * X_2$	1	21698	18145	18144.9	9.50	0.007
$X_3 * X_3$	1	14268	12745	12745.1	6.68	0.020
$X_4 * X_4$	1	18964	18964	18964.0	9.93	0.006
Interaction	6	67941	67941	11323.5	5.93	0.002
$X_1 * X_2$	1	36981	36981	36980.9	19.37	0.000 (S)
$X_1 * X_3$	1	907	907	906.9	0.47	0.501
$X_1 * X_4$	1	20710	20710	20709.8	10.85	0.005 (S)
$X_2 * X_3$	1	1441	1441	1440.8	0.75	0.398
$X_2 * X_4$	1	7047	7047	7047.2	3.69	0.073 (S)
$X_3 * X_4$	1	855	855	855.3	0.45	0.513
Residu Error	16	30547	30547	1909.2	-	-
Lack-of-Fit	10	30523	30523	3052.3	746.11	0.000
Pure Error	6	25	25	4.1	-	0.000
Total	30	207393	-	-	-	-

Based on TABLE 3 carried out the test procedures to second order model. This hypothesis test was used to test the truth of the regression model. the test if there is a free variable (including quadratic and interaction effects) significantly influence the response variable. The hypothesis being tested is $H_0: \beta_i=0$.

$$H_1: \text{ada } \beta_i \neq 0; i=1.2.3....k$$

According on TABLE 3. the test regression parameters simultaneously produce a p-value of 0.000. meaning that the p-value obtained is smaller than the significance level used in the amount of $\alpha=0:05$. Thus it was decided to reject H_0 and conclude that the independent variable significant effect on the response variable. so that the second-order model is unacceptable (TABLE 4).

Determination of stationary point

Based on the regression coefficients in TABLE 2 can be arranged matrix B and B as follows:

$$b = \begin{bmatrix} -30,3417 \\ 22,4800 \\ 1,5131 \\ 3,0543 \end{bmatrix} \text{ and } B = \begin{bmatrix} -31,9435 & 24,0380 & -3,7643 & 17,9886 \\ 24,0380 & -32,9085 & -4,7447 & -10,4934 \\ -3,7643 & -4,7447 & -27,5806 & -3,6557 \\ 17,9886 & -10,4934 & -3,6557 & -33,6431 \end{bmatrix}$$

In order to obtain a stationary point as follows:

$$x_0 = -\frac{B^{-1}b}{2} = \begin{bmatrix} -0,876403 \\ -0,210201 \\ 0,233981 \\ -0,383074 \end{bmatrix}$$

Thus, the response to a stationary point solution is obtained as follows

$$\hat{y} = \hat{\beta}_0 + \frac{1}{2}x_0'b = 362,10 + 10.5251 = 373,135$$

Furthermore, the stationary point can be restored to the true value, acquired conditions that provide the optimal solution is as follows:

TABLE 4. Results the optimum conditions of Cu with RSM.

Variable	Optimal value (with coding)	Optimal value (without coding)
X ₁ (Conc. calcein)	-0.876403	0.112360
X ₂ (pH)	-0.210201	6.78980
X ₃ (Potential)	0.233981	-0.876602
X ₄ (Time)	-0.383074	42.3385

Characteristics of response surface analysis

Furthermore, the stationary point can be restored to the true value, acquired conditions that provide the optimal solution is as follows:

To get an idea of the characteristics of the surface of the first response calculating eigenvalues (λ) of the matrix B and obtained:

$$\lambda = [-68,4096 \ -29,5170 \ -23,3413 \ -4,8078]$$

Fourth eigen values are negative, then the shape of the surface response is maximum. It can also be seen from the plot contour and response surface plot. By making constant two among the 4 factors were observed at a stationary point, then it may be possible to plot the contour and response surface as shown in FIG. 1a-1e below.

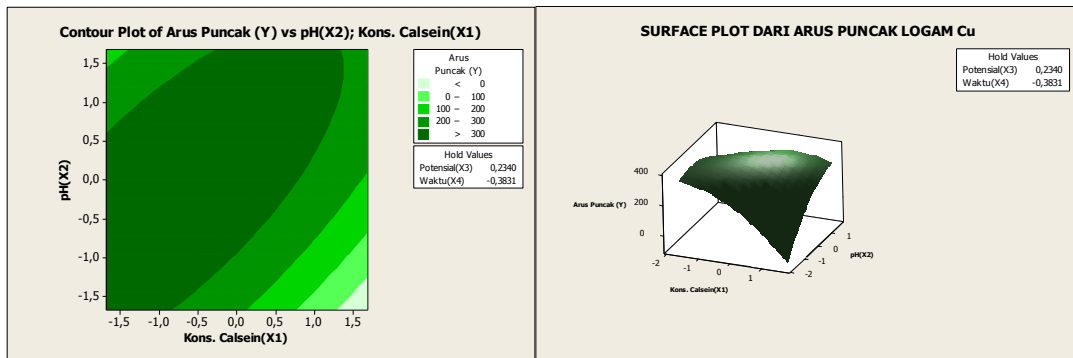


FIG. 1a. Countour and surface plot X3 and X4 in the stationary point. Countour plot and respon surface of peak current (Cu) on X₁ dan X₂.

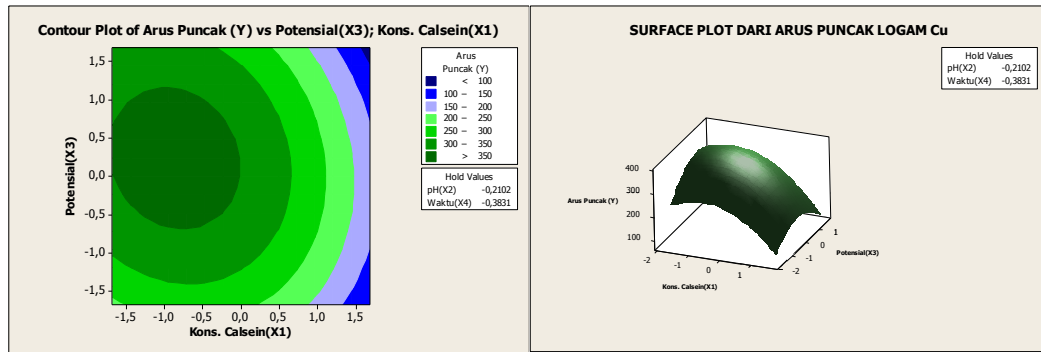


FIG. 1b. Countour and surface plot X₂ and X₄ in the stationary point. Countour plot and respon surface of peak current (Cu) on X₁ dan X₃.

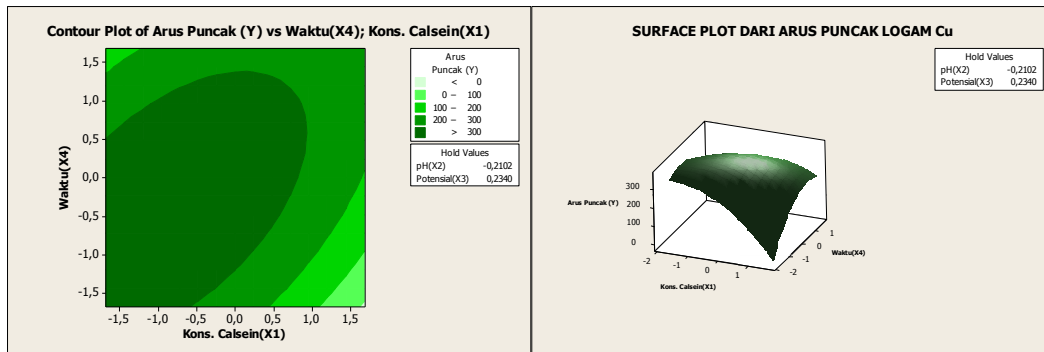


FIG. 1c. Countour and surface plot X₂ dan X₃ in the stationary point. Countour plot and respon surface of peak current (Cu) on X₁ dan X₄.

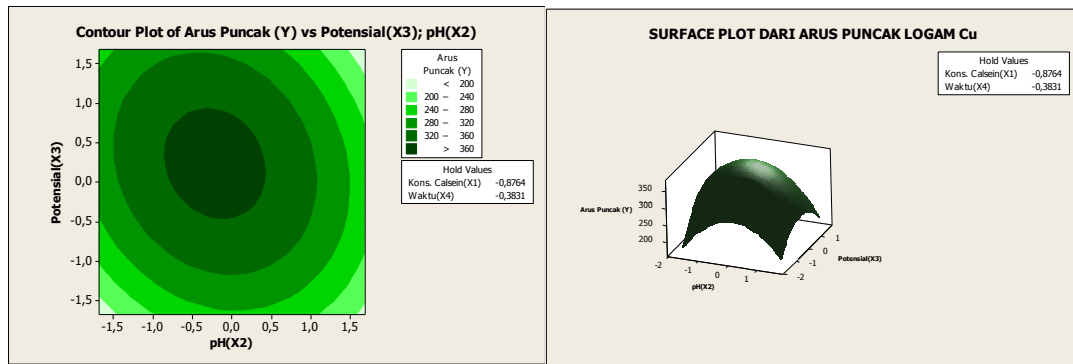


FIG. 1d. Countour and surface plot X_1 dan X_4 in the stationary point. Countour plot and respon surface of peak current (Cu) on X_2 dan X_3 .

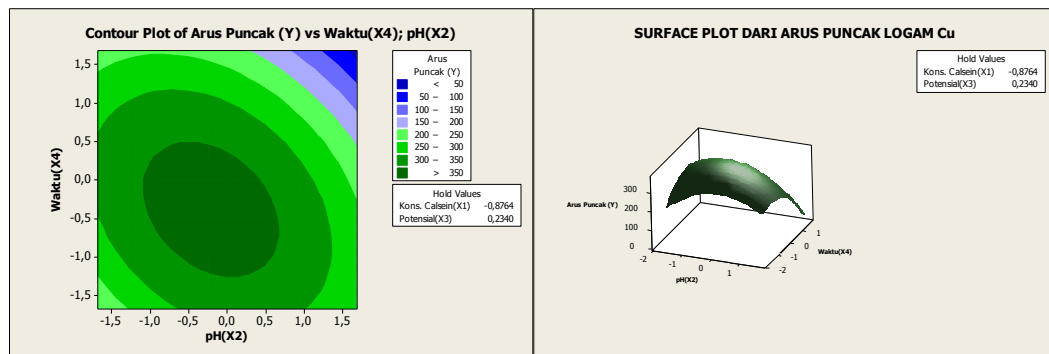


FIG. 1e. Countour and surface plot X_1 dan X_3 in the stationary point. Countour plot and respon surface of peak current (Cu) on X_2 dan X_4 .

Based on FIG. 1a-1e, it can be seen the range of values of each pair of variables that allegedly will give the maximum value. As described previously, that at the stationary point obtained an optimum response value. As an alternative to achieving the optimum response value, a combination of other values can be used as shown by the innermost circle of the contour plot. All pairs of points that are in this deepest circle are expected to produce a maximum response value with the following details:

- Each pair of points in the innermost circle of the plot between x_3 and x_4 will result in a predicted value of >100 response
- Each pair of points in the innermost circle of the plot between x_2 and x_4 will produce an estimated value of >110 response
- Each pair of points in the innermost circle of the plot between x_2 and x_3 will produce an estimated value of >110 response
- Each pair of points in the innermost circle of the plot between x_1 and x_4 will result in a predicted value of >100 response
- Each pair of points in the innermost circle of the plot between x_1 and x_3 will result in a predicted value of >100 response

- Each pairs of points in the innermost circle of the plot between x_1 and x_2 will produce an estimated value of >110 response.

It can be given the choice of alternatives to generate maximum response values, there is flexibility in determining the level of each factor that is thought to be capable of generating maximum response value.

Application method

To evaluate the validation of the proposed method for the analysis of real samples, the proposed procedure is applied to various samples such as fruit and vegetable samples (cabbage, broccoli, mustard greens, eggplant, apple, dragon fruit, melon and banana kepok. The Copper concentration in the sample can be seen in TABLE 6 and determined by the standard addition method using the recommended procedure under optimum conditions in the sample of fruit and vegetable.

TABLE 5. Comparison of methods of determination of copper in fruit and vegetable samples.

Sample	AdSV (mg/L)	AAS (mg/L)
Broccoli	3.781	1.161
Cauliflower	2.425	1.032
Eggplant	3.521	1.402
Mustard	5.223	1.134
Apples	3.023	1.387
Banana kepok	3.854	1.052
Dragon fruit	7.231	1.171
Melon	4.872	1.397

The results from TABLE 5 show that the AdSV method gives satisfactory results when compared to the atomic absorption spectrophotometer (AAS) method. Furthermore, determination of Copper metal in fruit and vegetable samples in AdSV by using calcein can be seen in TABLE 6.

TABLE 6. Determination of copper in fruit and vegetable samples by AdSV using calcein.

Sample	Cu (mg/L)
Broccoli	3.781
Cauliflower	2.425
Eggplant	3.521
Mustard	5.223
Apples	3.023
Banana kepok	3.854
Dragon fruit	7.231
Melon	4.872

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