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Bridging Virtual and Real: Testing Design of Experiment Procedures with Simulated and Experimental Data†

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Abstract: A virtual experiment conducted using the ICP-MS TuneSim software served as the basis for studies that compared the applicability of the central composite design with the Box-Behnken design in analytical chemistry applications. The insights gathered from these virtual experiments were used in real-life electroanalytical tests, including the determination of germanium and the studies of the antioxidant properties of herbal infusions. The experimental design and interpretation of the results were performed using Statistica software.

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1. Introduction

Optimisation of chemical and instrumental variables is a crucial stage of every analytical procedure as well-optimised procedures improve the precision, repeatability, and sensitivity, thus enhancing the accuracy of analysis. Design of experiments (DOE) is a statistical tool that allows the simultaneous investigation of multiple factors and interactions, reducing the total number of experiments needed to assess the roles of these variables in the results obtained [1]. Unfortunately, DOE procedures are not used routinely during the optimisation of instrumental and chemical factors of analytical procedures [2]. An analysis of data available in the Scopus database (keyword: stripping voltammetry) reveals that only 2.5% of the works contained information in their abstracts that the central composite design (CCD) was employed. This is probably because there are few sources that offer experimental data that allow a beginner user to test the DOE software and select the most suitable option for their needs.

The purpose of our work was to use a virtual experiment conducted using the ICP-MS TuneSim 0.7 software [3] to evaluate the applicability of different DOE procedures in the optimisation of analytical chemistry procedures. The software offered the user the possibility of generating measurement results for determinations of ^{115}In and ^{235}U, for different positions of the ICP torch (x, y, z), the power of the RF generator and the flow of the sample gas. Two of the most common designs, namely the Central Composite and Box-Behnken designs, were tested, and the results obtained were compared. The experience gained from the virtual experiments was intended to be transferred to real-life electroanalytical experiments, including the determination of the germanium and antioxidant properties of the herbal infusion.

2. Materials and Methods

For experiment planning (Central Composite Design (CCD); Box-Behnken Design (BBD), analysis of obtained results, and preparation of surface response plots, the Statistica 13.3 software package (TIBCO Software) was used.

Virtual experiments aimed at indium determination by ICP-MS were carried out using the ICP-MS TuneSim 0.7 programme [3].

Electrochemical studies aimed at the determination of germanium [4] and the determination of the antioxidant capacity of plant infusions were performed on an Autolab 204 analyser (Metrohm Autolab, Switzerland). Commercial glassy carbon disc electrodes (3 mm in diameter, Mineral, Poland) were used as working electrodes. Platinum wire and Ag/AgCl(3M KCl) were applied as auxiliary and reference electrodes. To record the voltammetric curves, the CV or DP mode (ΔE = 50 mV) was used. The solutions were stirred during the deposition step, which was followed by 5 s of equilibration.

Ginkgo biloba extracts were prepared by brewing 0.15 g of plant material and 50 g of distilled water in thermostatic quartz vessels.

3. Results and Discussion

3.1. Determination of indium by ICP – MS

Central Composite Design (CCD) and Box-Behnken Design (BBD) are powerful tools for optimising processes with multiple variables. During the optimisation, a mathematical model is created to represent the relationship between the response variables and the factors under study. The values predicted by this model can be viewed as approximations of the actual system behaviour, based on the experimental data. The optimisation process involves the following steps:

(i) Create an experimental design table that provides low, high, and central values for the variables (factors) being optimised;

(ii) Collect data according to the designed table;

(iii) Determine the equation that describes the relationship between the response and the independent variables;

(iv) Identify the optimal conditions for the response.

The design table for experiments, with variables coded as 0, +1, or -1 on a normalised scale for classic CCD, CCD with centred star points (CCD - CSP) and BBD, is presented as Table 1. The number of experimental runs (or trials) required for the CCD and BBD designs can be determined using Equations 1 and 2.

$$N = 2^k + 2k + N_0 \text{ (CCD)} \quad (1)$$

$$N = 2k(k-1) + N_0 \text{ (BBD)} \quad (2)$$

Where N is the total number of runs, k is the number of factors, N_0 is the number of centre points (replications at the centre of the design space).

When using CCD and BDD designs, experiments should be performed for various combinations of 1, -1, and 0 (for 3 variables: 14 measurements in CCD or 12 in BBD). Additionally, repetitions are required for the central points with coordinates (0,0,0). During the present studies, measurements were also taken for three additional central points to better estimate the repeatability of the measured values. While Table 1 presents the experiments of the ordered experiment, the measurements were taken in a random manner.

Table 1. Design table for CCD, CCD with centred star points (CCD – CSP), and BBD with variables coded as 0 for central, +1 for highest and -1 for lowest values. The blue data represents the star points of CCD. Additional optional central points are highlighted in grey.

No.	CCD			CCD-CSP			BBD		
	x	y	z	x	y	z	x	y	z
1	-1	-1	-1	-1	-1	-1	-1	-1	0
2	-1	-1	1	-1	-1	1	1	-1	0
3	-1	1	-1	-1	1	-1	-1	1	0
4	-1	1	1	-1	1	1	1	1	0

5	1	-1	-1	1	-1	-1	-1	0	-1
6	1	-1	1	1	-1	1	1	0	-1
7	1	1	-1	1	1	-1	-1	0	1
8	1	1	1	1	1	1	1	0	1
9	-1.68	0	0	-1	0	0	0	-1	-1
10	1.68	0	0	1	0	0	0	1	-1
11	0	-1.68	0	0	-1	0	0	-1	1
12	0	1.68	0	0	1	0	0	1	1
13	0	0	-1.68	0	0	-1	0	0	0
14	0	0	1.68	0	0	1	0	0	0
15	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	-	-	-

The ICP-MS TuneSim 0.7 software allowed the torch to be moved in three dimensions. The position in the x and y axes could be changed in a range from -4.5 to 4.5 mm and from -2.5 to 2.5 mm in the z axis. Other variables, such as argon flow (cool, auxiliary and sample) and generator power, remained constant at 15.5, 0.9, 0.2 L/min and 1000 W, respectively. The simulation was conducted with the guard electrode activated. The visual representation of the coded and real variables for CCD and BDD is shown in Figure 1.

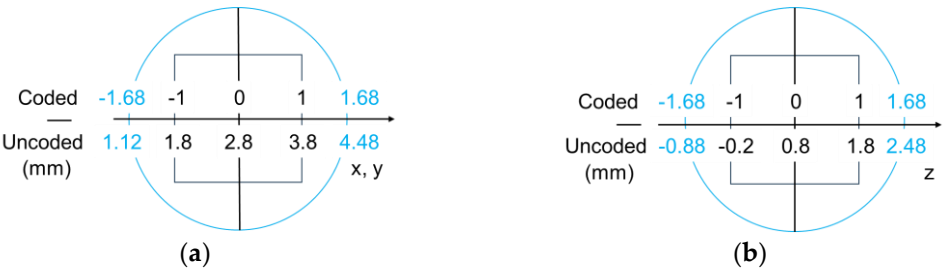
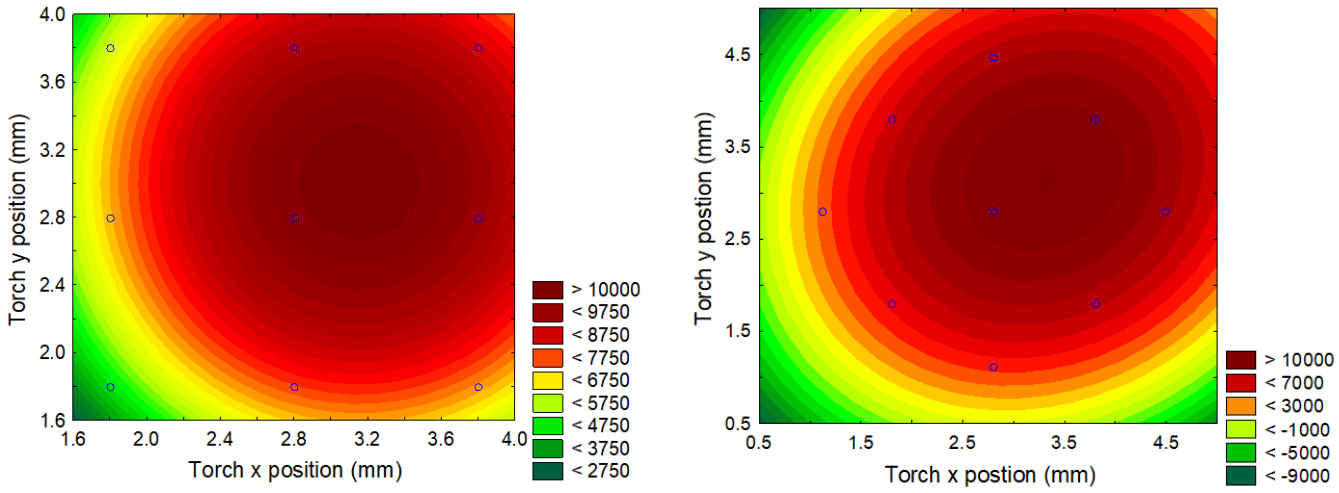


Figure 1. Coded and actual values of the ICP torch position on the x-, y- and z-axes used for BBD and CCD optimisation. The blue values indicate the values for the star points of CCD. These were rounded to decimal places during experiments, since the torch position could be adjusted in increments of 0.1 mm.

3.1.1. Optimization of ICP torch position employing Box-Behnken Design

Optimisation of the ICP torch position using BBD was performed using 3 separate design tables (BBD1-BBD3). The indium signal expressed as the count per second (cps) was collected five seconds after entering the torch position. The surface profile of the response surface obtained for the first of three experiments (BBD1) and z = 0.3 mm is presented in Figure 2a. Response surface profiles exhibit a well-defined maximum at the point with coordinates (3.2,3.0) expressed in mm. Comparison of predicted values with actual observed values confirms that the calculated model describes the relationships well within the experimental system. The results obtained for the remaining two experiments BBD2 and BBD3 were similar. The optimised coordinates of the ICP torch position, together with the predicted values of the indium signals, for all BBD experiments are presented in Table 2. The positions of the ICP torch obtained as a result of optimisation differed in the z-coordinate. The lowest z value obtained in the BBD2 experiment was correlated with the highest value of the indium signal. Experiments BBD1 and BBD3 yielded similar results, while experiment BBD2 provided the results, statistically higher than the others.



(**a**) (**b**)

Figure 2. (**a**,**b**) Response surface profiles of the intensity of the indium signal versus the position of the ICP torch x and y generated after optimisation of the variables using (**a**) BBD1 and (**b**) CCD designs constructed for the optimal position z (z = 0.3 mm (BBD1) or z = -0.9 mm (CCD)).

Table 2. ICP-MS torch position optimised for indium determination using BBD (three trials), CCD and CCD with centred star points (CCD – CSP) and intensity of In signal intensity calculated based on the model compiled with average intensities (n = 15) measured using optimal torch position.

Parameter		BBD1	BBD2	BBD3	CCD– CSP	CCD
Torch position	X (mm)	3.2	3.2	3.2	3.3	3.3
	Y (mm)	3.0	3.0	3.0	3.1	3.2
	Z (mm)	0.3	0.1	0.2	-0.9	-0.9
Intensity of ^{115}In signal (cps)	predicted	10463±346	10496±3472	10458±354	10837±1160	11043±1098
	measured	10417±43	10506±65	10432±54	11877±65	11879±69

3.1.2. Optimization of ICP torch position employing Central Composite Design

Experiments using CCD were conducted in two variants on the positioning of the star points. In the conventional approach, the star points were placed at ±1.68 units away from the central point, as shown in Table 1 and delineated in Figure 1 in blue. For the second variant, the method of centred star points (CCD – CSP) was used, where the star points were set at coordinates of ±1. The response surface profiles of CCD indicate pronounced maxima at coordinates (3.3,3.1,-0.9) for CCD (Figure 2b) and (3.3,3.2,-0.9) for CCD – CSP. The specified coordinates for the ICP torch position notably deviate in the z-coordinate compared to the data retrieved from BBD. In the BBD design process, it was observed that a lowered z-coordinate value resulted in elevated indium signals. During optimisation using the CCD approach, -0.9 mm was identified as the optimal value (see Table 2). Indium signal intensities closely correspond to the experimental data (Table 2). The indium signal intensities for the optimal torch positioning determined by both the CCD and CCD-CSP methods show negligible differences. However, they are, on average, 13.6% higher than signals acquired from torch positions optimised using BBD.

3.2. Determination of germanium by catalytic adsorptive striping voltammetry

After the studies using virtual data, the CCD was used to optimise the experimental parameters in real-life voltammetric measurements. The determination of germanium through catalytic adsorptive stripping voltammetry with a bismuth film electrode has previously been described [4]. Figure 3 shows the response surface profiles for the germanium peak current as a function of charge and plating potential (Figure 3a), as well as the correlation plot of predicted versus observed values (Figure 3b). The relationship between these values is described by the equation y=(0.926±0.070)x+(0.35±0.34), r^2=0.9264. A low

RMSE value of 0.22 indicates a good fit of the model to the observed values. A prominent maximum is observed for a solution containing 16.7 mM bismuth, with a plating potential of -1.156V, and electrolysis performed to exchange a charge of 42.8 mC. In previous studies [4], the peak of the CCD surface was outside the experimental domain. The optimal plating parameters included a plating potential of −0.9 V and other variables at their maximum investigated values (40 mM Bi(III) ion concentration, 50 mC). Following reoptimisation, the consumption of bismuth salt was reduced by using a less concentrated plating solution and a smaller electrolytic charge.

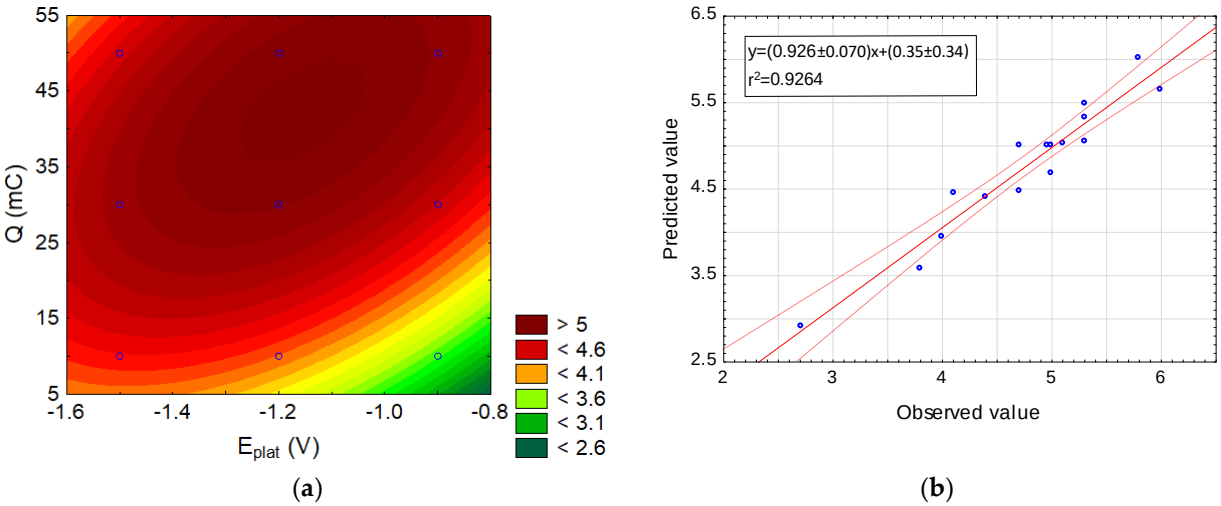
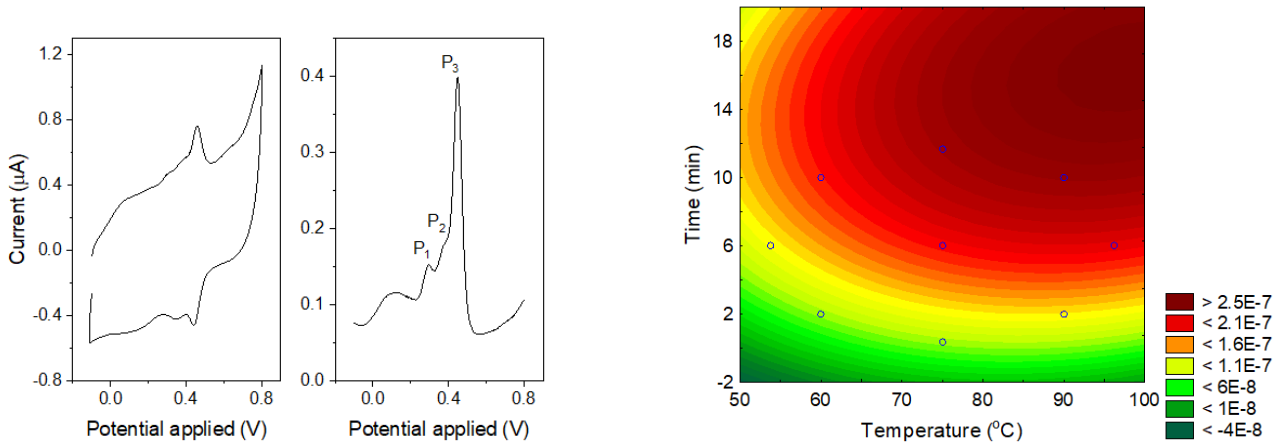


Figure 3. (**a**) CCD – CSP response surface profile of the germanium peak current versus charge and plating potential generated after optimisation of the bismuth plating parameters constructed for an optimal bismuth concentration equal to 16.7 mM. (**b**) Plot comparing the predictions from the design and experimental data for CCD. The 95% confidence band selected using a dash line. The peak currents were measured for differential pulse voltammograms recorded in solutions containing 30 nm Ge(IV), 0.05 M acetate buffer (pH of 4.4), 1 mM catechol, 1 mM of V(IV), and 1.5 mM of HEDTA after accumulation at E_{acc} = 0.4 V for t_{acc} = 30 s. Bismuth plating was performed in 0.34 M $HClO_4$.

3.3. Determination of antioxidant properties using voltammetry

Voltammetric procedures can be applied to determine the antioxidant properties of herbal infusions [5-7]. Figure 4a shows voltammograms recorded in a 20-fold diluted Ginkgo biloba extract, revealing three oxidation signals. These signals are formed as the oxidation of compounds to which plant extracts attribute their antioxidant properties occurs. To achieve the highest antioxidant properties of the infusion, parameters such as temperature and extraction time were optimised using CCD, as shown in Figure 4b. The maximum response surface profile is outside the experimental region selected by the blue circles, suggesting that for Ginkgo infusions, a relatively high temperature combined with a prolonged extraction time (temperature 98.5°C, time 16.5 min) is most appropriate [8].



(**a**) (**b**)

Figure 4. (**a**) CV and DPV voltammograms recorded in solution containing 20 times diluted Ginkgo biloba extract. (**b**) CCD response surface profile of P3 DPV peak current vs time and temperature of extraction. Supporting electrolyte: 0.15 M acetic acid, E_{acc} = 0 V, t_{acc} = 5 s, 0.1 V/s (CV).

4. Conclusions

Due to the ease of obtaining results from virtual experiments using ICP-MS TuneSim software [3], several DOE models were tested in a short period, identifying the model best suited to optimise analytical methods. For a design with three factors (x, y, z coordinates of the ICP torch position), virtual experiments revealed that the Central Composite Design (CCD) yielded superior results compared to the Box-Behnken design, without a significant increase in the number of measurements (19 for CCD vs. 18 for BBD). Ultimately, CCD was implemented in real-life voltammetric experiments, further solidifying its effectiveness. As a result of the use of CCD for the optimisation of the electrolytic process of producing bismuth electrodes for stripping voltammetry, it was possible to reduce the consumption of bismuth salt and to shorten the time for the electrolytic deposition of bismuth layers. Furthermore, optimising the extraction conditions of plant materials, combined with the recording of voltammetric curves, which allow a quick and precise assessment of their antioxidant properties, can be a decisive tool in the case of debates regarding the preparation conditions of plant infusions.

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Conflicts of Interest: The authors declare no conflict of interest.

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