# Experimental Design☆

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This is an update of G. Hanrahan, J. Zhu, S. Gibani, DG. Patil, Chemometrics and Statistics: Experimental Design, Editor(s): P. Worsfold, A. Townshend, C. Poole, *Encyclopedia of Analytical Science* (Second Edition), Elsevier, 2005, pp. 8–13, ISBN 9780123693976.



## Glossary

***Experimental design*** A specific set of experiments which are determined by a matrix that has a minimum of two factors and also a minimum of two levels, that must be developed to obtain the responses. The number of experiment and the levels of the factors studied depends of the matrix established by the chemometric tool. The experimental design simpler is the two-level full factorial design ($2^k$), where $k$ is equal at 2. The matrix of this experimental design has two levels ($+1$ and $-1$) and four experiments. The matrix of the experimental design ($3^k$) with two factors has three levels ($+1$, 0, $-1$) and nine experiments.

***Experimental domain*** The work range of a factor that is changed during the development of a procedure. These parameters must be defined with criteria because all the results and conclusions obtained by experimental design are limited by the domains of the factors. No conclusion can be inferred outside of the work range studied.

***Factors*** The independent variables of the experimental designs that can be changed independently of each other. During the optimization of a separation procedure employing solid phase extraction the factors studied were: kind and amount of the solid phase, nature and volume of the eluent, pH and shaking time. Nature of the solid phase and of the eluent are qualitative factors. Amount of the solid phase, pH, shaking time and amount of the solid phase are quantitative factors.

***Levels*** The different values that a factor is changed during the development of a procedure. They can be presented as coded or real values. The coded level is the value that it assumes in the geometric figure of the experimental design. The real level is the experimental value that the procedure is performed. During the development of a multivariate optimization all factors should have a minimum of two levels.

***Model*** Model of an experimental design is an equation that correlates the response of a design with the experimental factors investigated. The exponents demonstrate if the model is linear, quadratic or cubic, and the values and signals of the coefficients allow an interpretation of the influence of these factors on the response.

***Residual*** The difference between the observed and predicted value of the response for an experimental condition of the process investigated. When the experimental data are well fitted in the model, the residual values are low.

***Responses*** The dependent variables of the experimental designs that are the measured values of the results from experiments. These values are employed to establish the correlation models with the factors. Typical responses obtained in analytical procedures are absorbances, electrical signals, net emission intensities, residual carbon contents, extraction percentage of an analyte, and many others. The correlation models are established involving the factors (minimum of two) and only one response. However, most of the analytical procedures require the optimization of more than one response. So, for example, the optimization of strategies involving multi-element techniques such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP OES) and chromatographic separation processes provide more than one response. In these cases, the establishment of multiple responses is mandatorily necessary. These multiple responses obtained should be compatible with the goals of the optimization, prioritizing and/or minimizing individual

☆*Change History*: October 2018. SL. Costa Ferreira updated the text, references, and tables.

responses according to the proposed interests.[2] The tool more used to optimize multiple responses is the desirability function *D*, that was proposed in 1980 by Derringer and Suich.[3] In this, the main object is to find operating conditions that ensure compliance with the criteria of all the involved responses and, at the same time, provide the best value of compromise in the desirable joint response. The function is established in view of the experimental conditions which allow reach simultaneously the optimal value for all the evaluated variables. The desirability function has been widely employed in the optimization of analytical methods involving multi-element determinations.[4] Additionally, another multiple response function (MR) was proposed by Ferreira et al.[5] and has been used for optimization of analytical strategies involving multi-element determinations. The MR function is established by the average of the sum of the normalized responses for each analyte considering the highest value of these. Recently, a work reported a comparison between the MR and desirability functions during the employ of two-level full factorial design and Box Behnken matrix.[2]

## Introduction

Chemometrics is a discipline that manipulates data from chemical processes utilizing mathematics and statistic fundamentals. The advancement of the electronics and computer science have allowed a constant growth of Chemometrics, expanding the applications of this discipline in practically all sub-areas of chemistry. Fig. 1 shows the interaction of the Chemometrics with Statistics, Mathematics, Electronics, and Computer Sciences.[1] In this, it can also be seen "common sense" which reports the intellectual participation of the operator in the decisions making that occur during the development of the chemometric studies. This intellectual involvement with "common sense" is indispensable because several actions developed in optimization process are established by the operator, including the choice of the variables, definition of the experimental domains of these, critical evaluation of the results, among others. Additionally, all the information provided by the statistical programs should be evaluated with a critical sense, which makes the human participation indispensable in multivariate optimization processes.[1]

## Two-Level Full Factorial Design

The two-level full factorial design is the chemometric technique more employed by the chemists. The number of experiments ($N$) for this tool is defined by expression $N = 2^k$, where $k$ is the factor number.

The factorial design allows the calculation of the effects of the factors and their interactions by the Eq. (1), where $E_A$ represents the effect of factor $A$, and Y's are the responses obtained during the development of the experiments at positive ($Y_+$) and negative ($Y_-$) levels.

$$E_A = \frac{\Sigma\left(Y_{\text{at positive level}}\right)}{N/2} - \frac{\sum\left(Y_{\text{at negative level}}\right)}{N/2} \quad (1)$$

This tool also allows a preliminary evaluation of the factors based on the generation of linear models, which can be written for two factors as being:

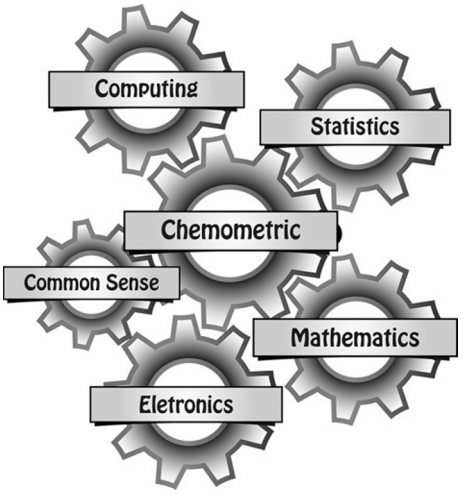


**Fig. 1** Interaction between chemometric and other sciences.

$$R = a + b\mathbf{A} + c\mathbf{B} + d\mathbf{AB} + \tag{2}$$

where ***R*** is the response, **A** and **B** are the factors, ☒ is the residual and *a*, *b*, *c*, and *d* are the model coefficients. For three factors the model generated, including **C** as the third factor is:

$$R = a + b\mathbf{A} + c\mathbf{B} + d\mathbf{C} + e\mathbf{AB} + f\mathbf{AC} + g\mathbf{BC} + \tag{3}$$

The coefficients of the factors and the interactions of the models correspond to half of the values of the effects of these factors and their interactions. By another hand, the independent term of the model "*a*" corresponds to the mean of the response values of the factorial design.

### Statistical Significance of the Effects of Factors and their Interactions

The effects of the factors and their interactions should be written as confidence intervals (**CI**), according to the Eq. (4):

$$\mathbf{CI} = \mathrm{Effect}_{\mathbf{F}} \pm S_{\mathrm{effect}} x\, t \tag{4}$$

where, **CI** is the confidence intervals, $\mathrm{Effect}_{\mathbf{F}}$ is calculated by the Eq. (1), $S_{\mathrm{effect}}$ represents the standard deviation of the effects of the factors and their interactions, and *t* is the Student's coefficient, which has been obtained in *t*-Table considering the degree of freedom of the replicates used for calculation of the estimated variance of the experimental error.

A factor or interaction have been considered statistically significant when its effect, expressed as a confidence interval (**CI**) does not include the value zero.[1,6]

### Determination of the Standard Deviation of the Effects by Using the Center Point

The standard deviation of the effects $S_{\mathrm{effect}}$, which has been calculated by the Eq. (5), where $S^2$ is the estimated variance of the experimental error, *N* is the number of different experiments, which is determined by $N = 2^k$, and *k* is the number of factors.

$$S_{\mathrm{effect}} = \left(\frac{4S^2}{N}\right)^{\frac{1}{2}} \tag{5}$$

The Eq. (6) expresses the calculation of the estimated variance of the experimental error, where $x_i$ is the individual values of the responses achieved, $x_o$ the average of the values of these responses and $(rn-1)$ the degree of freedom, considering *rn* as the number of replicates performed during the experiments.[1]

$$\mathrm{S}^2 = \frac{\sum (x_i - x_0)^2}{rn} - 1 \tag{6}$$

### The Standardized Effects and the Pareto Chart

The Pareto chart is a graphic of horizontal or vertical bars, frequently employed by the chemists for evaluation of results from experimental designs. In this, the effects of the factors and their interactions are presented as "standardized effects" (SE), which are calculated by the ratio between the "effect value" and its standard deviation obtained during the optimization. Also, a vertical line is drawn (on the horizontal bars) to show the factors and interactions that are effectively significant. This line on the *x*-axis corresponds to the critical value of the Student's t distribution, which is determined considering the degree of freedom involved in the calculation of the standard deviation of the effects and the confidence level established in the experimental work.[1]

$$SE = \frac{\mathrm{Effect\quad value}}{S_{\mathrm{effect}}} \tag{7}$$

### Curvature Test

Curvature test in two-level factorial designs involves the development of experiments at the central point of the factors to determine the experimental error and also to evaluate the influence of the factors on the response under these conditions. In this test, the curvature is calculated using the expression:

$$\mathrm{Curvature} = R_{FD} - R_{CP} \tag{8}$$

where $R_{FD}$ is the average of the responses obtained from experiments achieved by the factorial design, and $R_{CP}$ is the average of the responses obtained for the central point. The curvature can only be calculated if these averages expressed as confidence interval at 95% level are different. If the curvature is positive, there is a minimum response condition in the region of the central point of the factors. However, a negative curvature demonstrates that there is a maximum response condition close to the central point. If the

means of $R_{FD}$ and $R_{CP}$ expressed as the confidence interval are equal, the curvature is not statistically significant, and there is no a maximum or minimum condition for the response to the condition of the central point of the factors.[6,7]

### Application

The optimization step of an analytical method proposed for the determination of gallium in bauxite employing inductively coupled plasma optical emission spectrometry (ICP OES) was performed using a two-level full factorial design involving the factors: radio frequency power, hydrochloric acid concentration, and nebulizer gas flow rate.[8] Triplicate of the central point was performed in order to determine the experimental error. The results are presents in Table 1. So, the data obtained for the radio frequency power (RFP) were used to demonstrate the calculation of the standardized effect of this factor, being that the replicates of the center point were employed to determine the standard deviation of the effects. The results are shown in Eq. (10) and Eqs. (11) and (12).

## Response Surface Methodologies

The response surface designs are chemometric tools that allow the determination of the nominal values of the factors (maximum or minimum), also referred to critical conditions.[9,10] For two factors the quadratic model generated is, as Eq. (9):

$$R = a + b\mathbf{A} + c\mathbf{B} + d\mathbf{A^2} + e\mathbf{B^2} + f\mathbf{AB} + \quad (9)$$

where $R$ is the response, **A** and **B** are the factors, ($b$, $c$, $d$, $e$, and $f$) are the coefficients of the factors, $e$ the residual, and ($a$) an independent term.

**Table 1** Optimization of method for gallium determination using ICP OES

| *Experiment* | *[HCL]* | *Flow rate* | *RFP* | *Emission intensities* |
|---|---|---|---|---|
| 1 | −1 (0.50) | −1 (0.35) | −1 (1.00) | 5306 |
| 2 | 1 (3.00) | −1 (0.35) | −1 (1.00) | 4446 |
| 3 | −1 (0.50) | 1 (0.45) | −1 (1.00) | 4397 |
| 4 | 1 (3.00) | 1 (0.45) | −1 (1.00) | 3756 |
| 5 | −1 (0.50) | −1 (0.35) | 1 (1.30) | 5624 |
| 6 | 1 (3.00) | −1 (0.35) | 1 (1.30) | 4847 |
| 7 | -1 (0.50) | 1 (0.45) | 1 (1.30) | 6095 |
| 8 | 1 (3.00) | 1 (0.45) | 1 (1.30) | 5279 |
| 9 (C) | 0 (1.75) | 0 (0.40) | 0 (1.15) | 5377 |
| 10 (C) | 0 (1.75) | 0 (0.40) | 0 (1.15) | 5501 |
| 11 (C) | 0 (1.75) | 0 (0.40) | 0 (1.15) | 5454 |

$$\text{EffectPower } RF = \frac{(5.624 + 4.847 + 6.095 + 5.279)}{4} - \frac{(5.306 + 4.446 + 4.397 + 3.756)}{4} = +985 \quad (10)$$

$$s2 = \frac{[(5377 - 5444)2 + (5501 - 5444)2 + (5454 - 5444)2]}{(3 - 1)} = 3919 \quad (11)$$

$$\text{Seffect} = \left(\frac{4s2}{N}\right)^{\frac{1}{2}} = \left[\frac{(4 \times 3919)}{8}\right]^{\frac{1}{2}} = 44.27. \quad (12)$$

## Some Remarks of the RSM's Used by the Chemists

### Box Behnken Design

This methodology can be employed for process modeling of three or more factor, all always with three levels. Box Behnken presents a spherical and rotatable design, which viewed on a cube, it consists of the middle points of the edges and the central point. In Table 2 can be seen data about use of Box Behnken design as a chemometric tool.[1,9,11]

### Doehlert Matrix

The Doehlert matrix is a RSM that allows the process modeling requiring a low number of experiments. For two factors, this matrix is established with three and five levels. On the other hand, for three factors this matrix is defined with three, five and seven levels. This

**Table 2** Comparison between efficiencies of the response surface methodologies

| *Factor* | P | *Number of experiments required* | | | | *Efficiency(ϕ)* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *CCD* | *BBD* | *DM* | *3kD* | *CCD* | *BBD* | *DM* | *3kB* |
| 2 | 6 | 9 | – | 7 | 9 | 0.67 | – | 0.86 | 0.67 |
| 3 | 10 | 15 | 13 | 13 | 27 | 0.67 | 0.77 | 0.77 | 0.37 |
| 4 | 15 | 25 | 25 | 21 | 81 | 0.60 | 0.60 | 0.71 | 0.19 |
| 5 | 21 | 43 | 41 | 31 | 243 | 0.49 | 0.51 | 0.68 | 0.09 |
| 6 | 28 | 77 | 61 | 43 | 729 | 0.36 | 0.46 | 0.65 | 0.04 |

*p*, Number of coefficients of the quadratic model; *CCD*, central composite design; *BBD*, Box Behnken design; *DM*, Doehlert matrix; *3KD*, Full three level factorial design.
Ferreira, S. L. C.; Silva, M. M., Jr.; Felix, C. S. A.; Silva, D. L. F.; Santos, S. S.; Santos Neto, J. H.; Souza, C. T.; Cruz, R. A., Jr.; Souza, A. S. Multivariate Optimization Techniques in Food Analysis—A Review. *Food Chemistry* **2017**; doi. 10.1016/j.foodchem.2017.11.114.

methodology has no experiments with all factors with positive or negative levels, which is an advantage. Data about the application of the Doehlert matrix as RSM are shown in Table 2.[1,9,12]

### Central Composite Design

Central composite design (CCD) is a RSM that have been used for process modeling from two or more factors, generally involving five levels. The CCD consists of a two-level full factorial design, a star design, and the central point. One of the advantages of the CCD is that the full two-level factorial design can be performed preliminarily for a previous evaluation of the factors and afterward used in the SRM. The Table 2 shows information about the applicability of the CCD.[1,9]

### Three Level Factorial Design

This design has low applicability as SRM, considering a large number of experiments that are required, besides it contemplates experiments with all factors at the negative or positive level, which is an inconvenience. Some information of this SRM are presented in Table 2.[1,9]

### Efficiencies of the Response Surface Methodologies

In chemometrics, the efficiency parameter ($\phi$) was proposed for establishing a comparison between the SRM's. It is defined as being the number of coefficients in the estimated model divided by the number of experiments required by design. Table 2 shows a comparison between the four SRM's.[13] In it can be seen that the Doehlert matrix has a higher efficiency and the three level factorial design is unfeasible when the number of factors is higher than 2.[1]

### Robustness

Robustness has been defined as being the capacity of an analytical procedure to produce unbiased results when small changes in the experimental conditions are made voluntarily.[12,14–16] The robustness tests are important because sometimes the analyst needs to modify one or a few chemical and/or instrumental parameters of a procedure, and this can only be done if these changes do not affect the method's accuracy considering the previously optimized results. The two-level full factorial designs would be the most recommended for robustness evaluations of analytical methods.[14] However, for operational convenience, the fractional factorial designs are more often used. The Taguchi and Plackett-Burman[16] designs have been one of the most employed in robustness evaluations.

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