



Review

Response surface methodology (RSM) as a tool for optimization in analytical chemistry

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ABSTRACT

A review about the application of response surface methodology (RSM) in the optimization of analytical methods is presented. The theoretical principles of RSM and steps for its application are described to introduce readers to this multivariate statistical technique. Symmetrical experimental designs (three-level factorial, Box–Behnken, central composite, and Doehlert designs) are compared in terms of characteristics and efficiency. Furthermore, recent references of their uses in analytical chemistry are presented. Multiple response optimization applying desirability functions in RSM and the use of artificial neural networks for modeling are also discussed.

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1. Introduction

Optimizing refers to improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it. The term *optimization* has been commonly used in analytical chemistry as a means of discovering conditions at which to apply a procedure that produces the best possible response [1].

Traditionally, optimization in analytical chemistry has been carried out by monitoring the influence of one factor at a time on an experimental response. While only one parameter is changed, others are kept at a constant level. This optimization technique is called one-variable-at-a-time. Its major disadvantage is that it does not include the interactive effects among the variables studied. As a consequence, this technique does not depict the complete effects of the parameter on the response [2]. Another disadvantage of the one-factor optimization is the increase in the number of experiments necessary to conduct the research, which leads to an increase of time and expenses as well as an increase in the consumption of reagents and materials.

In order to overcome this problem, the optimization of analytical procedures has been carried out by using multivariate statistic techniques. Among the most relevant multivariate techniques used in analytical optimization is response surface methodology (RSM). Response surface methodology is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical previsions. It can be well applied when a response or a set of responses of interest are influenced by several variables. The objective is to simultaneously optimize the levels of these variables to attain the best system performance.

Before applying the RSM methodology, it is first necessary to choose an experimental design that will define which experiments should be carried out in the experimental region being studied. There are some experimental matrices for this purpose. Experimental designs for first-order models (e.g., factorial designs) can be used when the data set does not present curvature [3]. However, to approximate a response function to experimental data that cannot be described by linear functions, experimental designs for quadratic response surfaces should be used, such as three-level factorial, Box–Behnken, central composite, and Doehlert designs.

The present paper discusses the use of RSM for optimization in analytical chemistry. First, its basic principles are presented. Then, the approach to the applications of its more frequently used second-order experimental designs is broached, as well as the optimization of procedures that generate multiple responses.

2. Definition of some terms

Before beginning the discussion on the applications of response surface in the optimization of analytical methods, it is pertinent to introduce and define some key terms. Examples are also presented to illustrate each term.

Experimental domain is the experimental field that must be investigated. It is defined by the minimum and maximum limits of the experimental variables studied.

Experimental design is a specific set of experiments defined by a matrix composed by the different level combinations of the variables studied. Doehlert is an example of a second-order experimental design. This design defines a specific set of combinations for the levels of variables that must be applied experimentally to obtain the responses.

Factors or independent variables are experimental variables that can be changed independently of each other. Typical independent variables comprise the pH, temperature, reagents concentration, microwave irradiation time, flow rate, atomization temperature, and elution strength, among others.

Levels of a variable are different values of a variable at which the experiments must be carried out. The variable pH, for example, can be investigated at five levels: 4, 5, 6, 7 and 8 in the optimization of a spectrophotometric method.

Responses or dependent variables are the measured values of the results from experiments. Typical responses are the analytical signal (absorbance, net emission intensity, and electrical signal), recovery of an analyte, resolution among chromatographic peaks, percentage of residual carbon, and final acidity, among others.

Residual is the difference between the calculated and experimental result for a determinate set of conditions. A good mathematical model fitted to experimental data must present low residuals values.

3. Theory and steps for RSM application

Response surface methodology was developed by Box and collaborators in the 50s [4,10]. This term was originated from the graphical perspective generated after fitness of the mathematical model, and its use has been widely adopted in texts on chemometrics. RSM consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design. Toward this objective, linear or square polynomial functions are employed to describe the system studied and, consequently, to explore (modeling and displacing) experimental conditions until its optimization [5].

Some stages in the application of RSM as an optimization technique are as follows: (1) the selection of independent variables of major effects on the system through screening studies and the delimitation of the experimental region, according to the objective of the study and the experience of the researcher; (2) the choice of the experimental design and carrying out the experiments according to the selected experimental matrix; (3) the mathematic–statistical treatment of the obtained experimental data through the fit of a polynomial function; (4) the evaluation of the model's fitness; (5) the verification of the necessity and possibility of performing a displacement in direction to the optimal region; and (6) obtaining the optimum values for each studied variable.

3.1. Screening of variables

Numerous variables may affect the response of the system studied, and it is practically impossible to identify and control the small contributions from each one. Therefore, it is necessary to select those variables with major effects. Screening designs should be carried out to determine which of the several experimental variables and their interactions present more significant effects. Full or fractional two-level factorial designs may be used for this objective principally because they are efficient and economical [2].

3.2. Choice of the experimental design

The simplest model which can be used in RSM is based on a linear function. For its application, it is necessary that the responses obtained are well fitted to the following equation:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \varepsilon, \quad (1)$$

where k is the number of variables, β_0 is the constant term, β_i represents the coefficients of the linear parameters, x_i represents the variables, and ε is the residual associated to the experiments.

Therefore, the responses should not present any curvature. To evaluate curvature, a second-order model must be used. Two-level factorial designs are used in the estimation of first-order effects, but they fail when additional effects, such as second-order effects, are significant. So, a central point in two-level factorial designs can be used for evaluating curvature. The next level of the polynomial model should contain additional terms, which describe the interaction between the different experimental variables. This way, a model for a second-order interaction presents the following terms:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{1 \leq i \leq j} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

where β_{ij} represents the coefficients of the interaction parameters.

In order to determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i \leq j} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where β_{ii} represents the coefficients of the quadratic parameter.

To estimate the parameters in Eq. (3), the experimental design has to assure that all studied variables are carried out at in at least three factor levels. Thus, two modeling, symmetrical response surface designs are available. Among the more known second-order symmetrical designs are the three-level factorial design, Box–Behnken design, central composite design, and Doehlert design. These symmetrical designs differ from one another with respect to their selection of experimental points, number of levels for variables, and number of runs and blocks. These experimental matrices are presented and discussed in Section 4.

3.2.1. Codification of the levels of the variable

Codification of the levels of the variable consists of transforming each studied real value into coordinates inside a scale with dimensionless values, which must be proportional at its localization in the experimental space. Codification is of concern because it enables the investigation of variables of different orders of magnitude without the greater influencing the evaluation of the lesser.

The following equation can be applied to transform a real value (z_i) into a coded value (x_i) according to a determinate experimental design:

$$x_i = \left(\frac{z_i - z_i^0}{\Delta z_i} \right) \beta_d \quad (4)$$

where Δz_i is the distance between the real value in the central point and the real value in the superior or inferior level of a variable, β_d is the major coded limit value in the matrix for each variable, and z_i^0 is the real value in the central point.

3.3. Mathematical–statistical treatment of data

After acquiring data related to each experimental point of a chosen design, it is necessary to fit a mathematical equation to describe the behavior of the response according to the levels of values studied. In other words, there must be estimates of the b parameters of Eqs. (1)–(3). Therefore, in matrix notation, Eqs. (1)–(3) can be represented as

$$y_m X_i = X_m X_n b_n X_1 + e_m X_1, \quad (5)$$

where y is the response vector, X is the matrix of the chosen experimental design, b is the vector constituted by the parameters of the

model, e is the residual, and m and n represent the numbers of lines and columns from the matrices, respectively.

Eq. (5) is solved by using a statistical approach called the method of least square (MLS) [6]. MLS is a multiple regression technique used to fit a mathematical model to a set of experimental data generating the lowest residual possible. After mathematical transformations of Eq. (5), a vector b containing the parameters can be obtained by the following equation:

$$b_{n-1} = (X_{n-m}^T X_{m-n})^{-1} (X_{n-m}^T y_{m-i}) \quad (6)$$

Eq. (6) is used in the construction of the response surface that describes the behavior of the response in the experimental domain. The great advantage of Eq. (6) is the low computational cost necessary to determine the b coefficients.

In the LSM, it is assumed that errors present a random distribution profile with a zero mean and a common unknown variance and that these errors are independent of each other. In this way, the variance estimate to each component of vector b is commonly obtained by authentic repetitions of the central point according to Eq. (7):

$$\hat{V}(b)_{n-n} = (X_{n-m}^T X_{m-n})^{-1} s^2 \quad (7)$$

Thus, extracting the square root for each component of $\hat{V}(b)$ leads to obtaining the standard errors for the b coefficients that compose the equation of the response surface, allowing the evaluation of its significance.

3.4. Evaluation of the fitted model

The mathematical model found after fitting the function to the data can sometimes not satisfactorily describe the experimental domain studied. The more reliable way to evaluate the quality of the model fitted is by the application of analysis of variance (ANOVA). The central idea of ANOVA is to compare the variation due to the treatment (change in the combination of variable levels) with the variation due to random errors inherent to the measurements of the generated responses [7]. From this comparison, it is possible to evaluate the significance of the regression used to foresee responses considering the sources of experimental variance.

In ANOVA, the evaluation of data set variation is made by studying its dispersion. The evaluation of the deviation (d_i) that each observation (y_i) or its replicates (y_{ij}) present in relation to the media (\bar{y}), or, more precisely, the square of this deviation, is presented in Eq. (8):

$$d_i^2 = (y_{ij} - \bar{y})^2 \quad (8)$$

The sum of the square for all observation deviations in relation to the media is called the total sum of the square (SS_{tot}); it can be dismembered in the sum of the square due to the fitted mathematical model, that is, due to regression (SS_{reg}), and in the sum of the square due to residuals generated by the model (SS_{res}), as shown below:

$$SS_{tot} = SS_{reg} + SS_{res} \quad (9)$$

As replicates of the central point are made, it is possible to estimate the pure error associated with repetitions. Thus, the sum of the square for residuals can be dismembered into two more parcels: the sum of the square due to pure error (SS_{pe}) and the sum of the square due the lack of fit (SS_{lof}), as shown below:

$$SS_{res} = SS_{pe} + SS_{lof} \quad (10)$$

When the division of the sum of the square for each source of variation (total, regression, residual, lack of fit, and pure error) is made by its respective numbers of degrees of freedom (d.f.), the

Table 1
Analysis of variance for fitted mathematical model to an experimental data set using multiple regression

| Variation source | Sum of the square | Degree of freedom | Media of the square |
|------------------|--|-------------------|-----------------------------------|
| Regression | $SS_{reg} = \sum_i^m \sum_j^{n_i} (\hat{y}_i - \bar{y})^2$ | $p - 1$ | $MS_{reg} = \frac{SS_{reg}}{p-1}$ |
| Residuals | $SS_{res} = \sum_i^m \sum_j^{n_i} (y_{ij} - \hat{y}_i)^2$ | $n - p$ | $MS_{res} = \frac{SS_{res}}{n-p}$ |
| Lack of fit | $SS_{lof} = \sum_i^m \sum_j^{n_i} (\hat{y}_i - \bar{y}_i)^2$ | $m - p$ | $MS_{lof} = \frac{SS_{lof}}{m-p}$ |
| Pure error | $SS_{pe} = \sum_i^m \sum_j^{n_i} (y_{ij} - \bar{y}_i)^2$ | $n - m$ | $MS_{pe} = \frac{SS_{pe}}{n-m}$ |
| Total | $SS_{tot} = \sum_i^m \sum_j^{n_i} (y_{ij} - \bar{y})^2$ | $n - 1$ | |

n_i , number of observations; m , total number of levels in the design; p , number of parameter of model; \hat{y}_i , estimated value by the model for the level i ; \bar{y} , overall media; y_{ij} , replicates performed in each individual levels; \bar{y}_i , media of replicates performed in the same set of experimental conditions.

“media of the square” (MS) are obtained. The numbers of degree of freedom for these sources of variation are calculated by the expressions presented in the third column of Table 1, where p represents the number of coefficients of the mathematical model, n represents the number of total observations, and m represents the numbers of levels used in the investigation. Equations related to the source of variations for the calculation of SSs and MSs are also presented in Table 1 [5,10].

The *significance of regression* can be evaluated by the ratio between the media of the square of regression (MS_{reg}) and the media of the square of residuals (MS_{res}) and by comparing these variation sources using the Fisher distribution (F test), taking into account its respective degrees of freedom associated to regression (v_{reg}) and to residual (v_{res}) variances:

$$\frac{MS_{reg}}{MS_{res}} \approx F_{v_{reg}, v_{res}} \quad (11)$$

Thus, a statistically significant value for this ratio must be higher than the tabulated value for F . This is an indication that the mathematical model is well fitted to the experimental data.

Another way to evaluate the model is the *lack of fit test*. If the mathematical model is well fitted to the experimental data, MS_{lof} should reflect only the random errors inherent to the system. Additionally, MS_{pe} is also an estimate of these random errors, and it is assumed that these two values are not statistically different. This is the key idea of the lack of fit test. It is possible to use the F distribution to evaluate if there is some statistical difference between these two media, in the same way that the significance of regression was verified:

$$\frac{MS_{lof}}{MS_{pe}} \approx F_{v_{lof}, v_{pe}} \quad (12)$$

where, v_{lof} and v_{pe} are, respectively, the degree of freedom associated with the lack of fit and the pure error variances. If this ratio is higher than the tabulated value of F , it is concluded that there is evidence of a lack of fit and that the model needs to be improved. However, if the value is lower than the tabulated value, the model fitness can be considered satisfactory. To apply a lack of fit test, the experimental design must be performed with authentic repetitions at least in its central point.

In short, a model will be well fitted to the experimental data if it presents a significant regression and a non-significant lack of fit. In other words, the major part of variation observation must be described by the equation of regression, and the remainder of the variation will certainly be due to the residuals. Most variation related to residuals is due to pure error (random fluctuation of measurements) and not to the lack of fit, which is directly related to the model quality [8,9].

The visual inspection of the residual graphs can also generate valuable information about the model suitability. Thus, if the mathematical model is well fitted, its graph of residuals presents a behavior that suggests a normal distribution. If the model generates

larger residuals, it is not adequate to make precise inferences about the data behavior in the studied experimental area. Moreover, if the model needs some other term, the residual graph will present a behavior that indicates the kind of term that must be added to the model [10].

3.5. Determination of the optimal conditions

The surfaces generated by linear models can be used to indicate the direction in which the original design must be displaced in order to attain the optimal conditions. However, if the experimental region cannot be displaced due to physical or instrumental reasons, the research must find the best operational condition inside the studied experimental condition by visual inspection.

For quadratic models, the critical point can be characterized as maximum, minimum, or saddle. It is possible to calculate the coordinates of the critical point through the first derivate of the mathematical function, which describes the response surface and equates it to zero. The quadratic function obtained for two variables as described below is used to illustrate the example:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (13)$$

$$\frac{\partial y}{\partial x_1} = b_1 + 2b_{11}x_1 + b_{12}x_2 = 0 \quad (14)$$

$$\frac{\partial y}{\partial x_2} = b_2 + 2b_{22}x_2 + b_{12}x_1 = 0 \quad (15)$$

Thus, to calculate the coordinate of the critical point, it is necessary to solve the first grade system formed by Eqs. (14) and (15) and to find the x_1 and x_2 values.

The visualization of the predicted model equation can be obtained by the surface response plot. This graphical representation is an n -dimensional surface in the $(n+1)$ -dimensional space. Usually, a two-dimensional representation of a three-dimensional plot can be drawn. Thus, if there are three or more variables, the plot visualization is possible only if one or more variables are set to a constant value. Fig. 1 illustrates some profile for the quadratic response surface plot in the optimization of two variables. Fig. 1(a and b) represents surfaces where the maximum point is located inside the experimental region. It is interesting to note that, in surface shown in Fig. 1(b), there is a plateau in relation to variable x_2 , indicating that variation of its levels does not affect the studied system. Surface shown in Fig. 1(c) shows that the maximum point is outside the experimental region and that it is necessary to displace, if possible, the initial design to attain it. The surface shown in Fig. 1(d) presents a minimum point, and that shown in Fig. 1(e) presents a saddle point as the critical point. The saddle point is an inflexion point between a relative maximum and a relative minimum. If the purpose is to obtain a maximum or minimum response to a studied system, the saddle point coordinates do not serve as optimal values. Again, it is possible to find the optimum region through visual inspection of the surfaces.

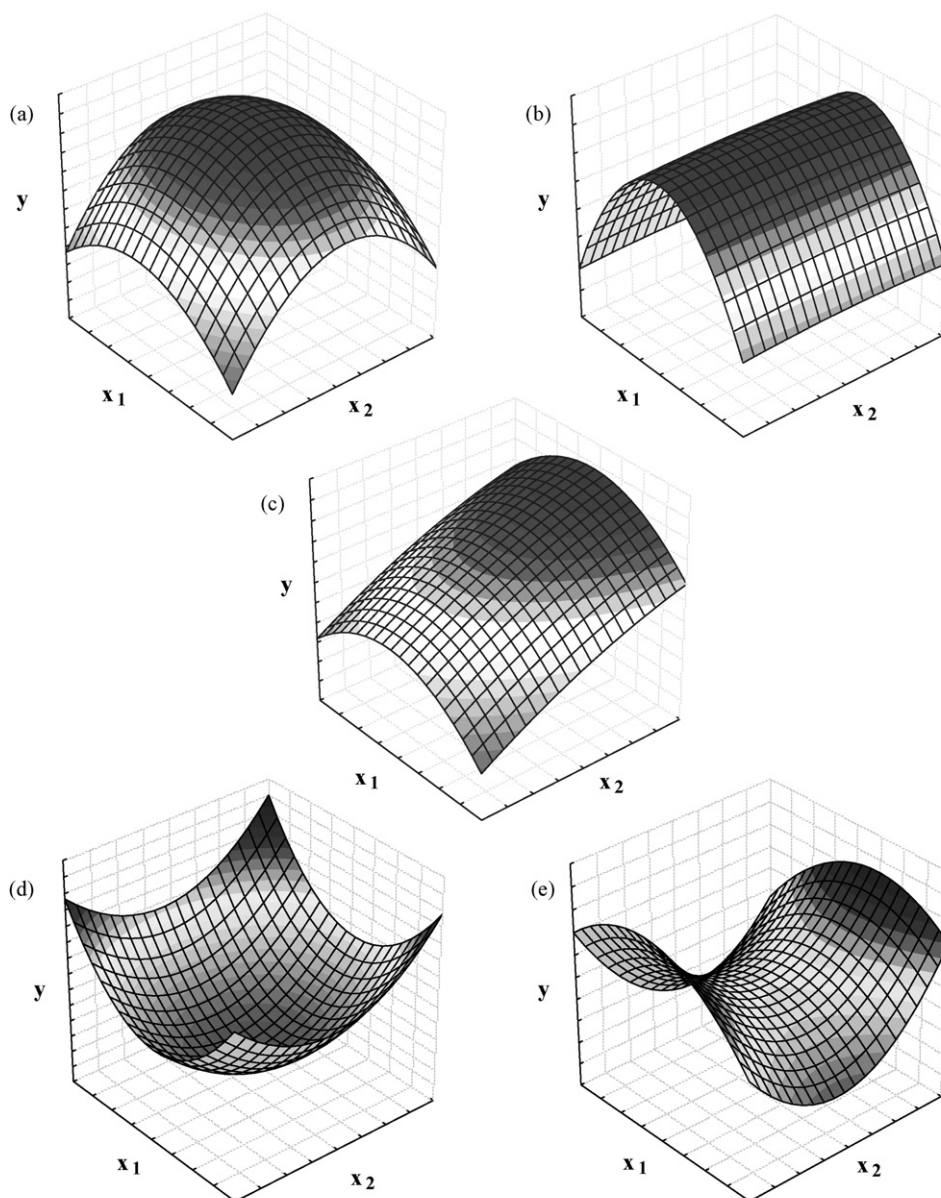


Fig. 1. Some profiles of surface response generated from a quadratic model in the optimization of two variables. (a) maximum, (b) plateau, (c) maximum outside the experimental region, (d) minimum, and (e) saddle surfaces.

4. Symmetrical second-order experimental designs and their applications in analytical chemistry

4.1. Full three-level factorial designs

Full three-level factorial design is an experimental matrix that has limited application in RSM when the factor number is higher than 2 because the number of experiments required for this design (calculated by expression $N=3^k$, where N is experiment number and k is factor number) is very large, thereby losing its efficiency in the modeling of quadratic functions. Because a complete three-level factorial design for more than two variables requires more experimental runs than can usually be accommodated in practice, designs that present a smaller number of experimental points, such as the Box–Behnken, central composite, and Doehlert designs, are more often used [11]. However, for two variables, the efficiency is comparable with designs such as central composite [12].

Fig. 2(a and b) shows the representation of the three-level factorial designs for the optimization of two and three variables, respectively. Table 2(a) shows the experimental matrix for the optimization of two variables using this design.

The majority of applications of three-level factorial designs are in the area of chromatography. Table 3 shows some works in which this experimental design was used.

4.2. Box–Behnken designs

Box and Behnken [13] suggested how to select points from the three-level factorial arrangement, which allows the efficient estimation of the first- and second-order coefficients of the mathematical model. These designs are, in this way, more efficient and economical than their corresponding 3^k designs, mainly for a large number of variables.

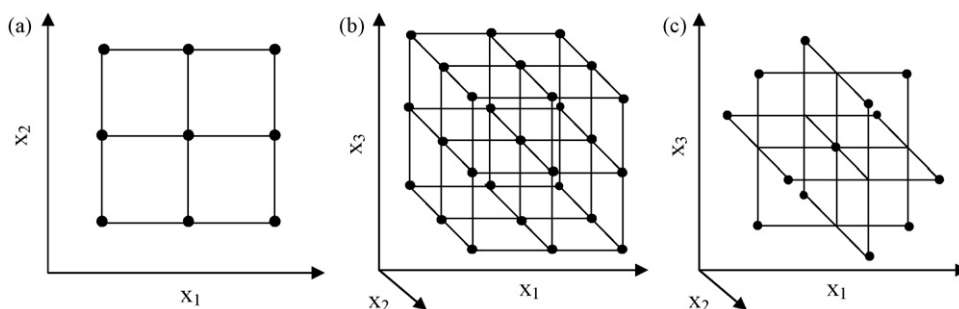


Fig. 2. Experimental designs based on the study of all variables in three levels: three-level factorial design for the optimization of (a) two variables and (b) three variables and (c) Box–Behnken design for the optimization of three variables.

Table 2

Some experimental matrices for designs based on variables study in three levels: (a) three-level factorial design for two variables and (b) Box–Behnken design for three variables matrices

| (a) | | (b) | | |
|-------|-------|-------|-------|-------|
| x_1 | x_2 | x_1 | x_2 | x_3 |
| –1 | –1 | –1 | –1 | 0 |
| –1 | 0 | 1 | –1 | 0 |
| –1 | 1 | –1 | 1 | 0 |
| 0 | –1 | 1 | 1 | 0 |
| 0 | 0 | –1 | 0 | –1 |
| 0 | 1 | 1 | 0 | –1 |
| 1 | –1 | –1 | 0 | 1 |
| 1 | 0 | 1 | 0 | 1 |
| 1 | 1 | 0 | –1 | –1 |
| | | 0 | 1 | –1 |
| | | 0 | –1 | 1 |
| | | 0 | 1 | 1 |
| | | 0 | 0 | 0 |

In Box–Behnken designs [14,15], the experimental points are located on a hypersphere equidistant from the central point, as exemplified for a three-factor design in Fig. 2(c). Its principal characteristics are:

- (1) requires an experiment number according to $N = 2k(k-1) + c_p$, where k is the number of factors and (c_p) is the number of the central points;
- (2) all factor levels have to be adjusted only at three levels (–1, 0, +1) with equally spaced intervals between these levels.

Table 3

Some applications of three-level factorial design in analytical chemistry

| Analytes | Samples | Analytical technique | Objective of study | Ref. |
|---|--|--------------------------------|--|------|
| Caffeine, theobromine and theophylline | Coffee, tea and human urine | Reversed-phase HPLC | Improving the chromatographic resolution among these three substances | [24] |
| Niacin | Fresh and dry-cured pork products | Ion chromatography | Optimizing the mobile phase composition | [25] |
| Anionic, cationic, and neutral drugs | Pharmaceutical formulations | Electrokinetic chromatography | Stabilising the effects of the sodium dodecyl sulfate and 2-propanol concentration in the separation of these analytes | [26] |
| Clothiapine, clozapine, olanzapine, and quetiapine | Pharmaceutical formulations | Capillary zone electrophoresis | Development of a method for separation of these four atypical antipsychotics | [27] |
| Sulfonamides | Foodstuffs | HPLC | Developing a molecularly imprinted polymer for separation of the analytes | [28] |
| Candesartan, eprosartan, irbesartan, losartan potassium, telmisartan, and valsartan | Pharmaceutical formulations | Capillary zone electrophoresis | Optimizing the separation of these angiotensin-II-receptor antagonists | [29] |
| Underivatized phenol and cresols | Soil samples with a high content of carbon | GC | Optimizing the supercritical fluid extraction of these analytes | [30] |
| Copper | Petroleum condensate | GF AAS | Developing a method for the direct determination of analyte using detergentless microemulsions | [31] |

Fig. 2(c) presents the Box–Behnken design for three-variable optimization with its 13 experimental points. In comparison with the original 3^3 design with 27 experiments (Fig. 2(b)), it is noted that this design is more economical and efficient. Table 2(b) presents the coded values to the application of this design for three variables.

This experimental design has been applied for the optimization of several chemical and physical processes; however, its application in analytical chemistry is still much smaller in comparison with central composite design. Table 4 shows some applications of the Box–Behnken design in analytical chemistry.

4.3. Central composite design

The central composite design was presented by Box and Wilson [16]. This design consists of the following parts: (1) a full factorial or fractional factorial design; (2) an additional design, often a star design in which experimental points are at a distance α from its center; and (3) a central point. Fig. 3(a and b) illustrates the full central composite design for optimization of two and three variables.

Full uniformly routable central composite designs present the following characteristics:

- (1) require an experiment number according to $N = k^2 + 2k + c_p$, where k is the factor number and (c_p) is the replicate number of the central point;
- (2) α -values depend on the number of variables and can be calculated by $\alpha = 2^{(k-p)/4}$. For two, three, and four variables, they are, respectively, 1.41, 1.68, and 2.00;
- (3) all factors are studied in five levels ($-\alpha, -1, 0, +1, +\alpha$).

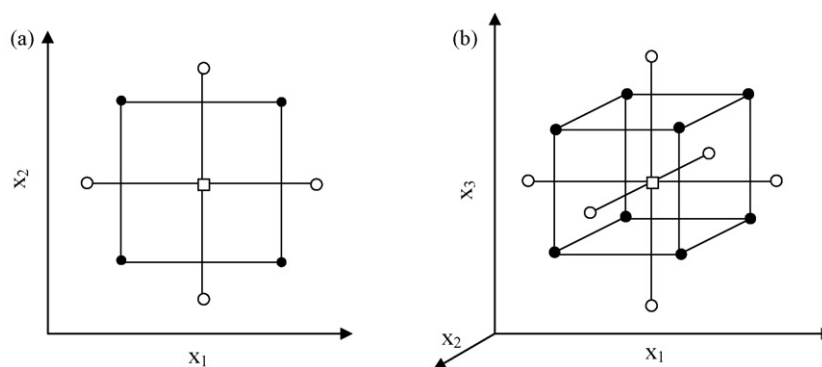


Fig. 3. Central composite designs for the optimization of: (a) two variables ($\alpha = 1.41$) and (b) three variables ($\alpha = 1.68$). (●) Points of factorial design, (○) axial points and (□) central point.

Fig. 3(a and b) shows representations of central composite designs for two- and three-variable optimization, respectively. Table 5(a and b) presents the coded values of the experimental matrices for the application of these designs.

Many applications of the central composite design in the optimization of analytical procedures can be found in the literature. Table 6 shows a limited number of applications as recent exam-

ples of the utilization of this design in some areas of analytical chemistry.

4.4. Doehlert design

Developed by Doehlert [17], the design is a practical and economical alternative in relation to other second-order experimental

Table 4
Some applications of Box–Behnken design in analytical chemistry

| Analytes | Samples | Analytical technique | Objective of the study | Ref. |
|---|----------------------------|--|---|------|
| Aliphatic aldehydes | Potato crisps | HPLC | Establishing the optimum conditions for the derivatization reaction of the analytes with 2,4-dinitrophenylhydrazine | [32] |
| Alprenolol, oxprenolol, promethazine and propranolol | Human serum albumin | Affinity electrokinetic chromatography | Optimization of the chiral separation of these four drugs | [33] |
| Cadmium | Drinking water | FAAS | Optimizing an on-line pre-concentration system using knotted reactor | [34] |
| Organochlorine pesticides | Sediments | GC | Optimizing a microwave-assisted extraction method for the extraction of persistent pesticides | [35] |
| Neuropeptides | Biological | Capillary zone electrophoresis | Optimizing the main electrophoretic parameters involved in the analytes separation | [36] |
| Lead | Waters | ICP OES | Optimizing a flow injection system for the on-line pre-concentration of these metal using silica gel functionalized with methylthiosalicylate | [37] |
| Atenolol, sotalol, betaxolol, and metoprolol | Non-aqueous | Capillary electrophoresis | Optimizing the separation of these four beta-blocking drug substances | [38] |
| Captopril | Tablets of pharmaceuticals | HPLC | Optimizing the chromatographic determination of this analyte | [39] |
| Sulphonamides, dihydrofolate reductase inhibitors and beta-lactam antibiotics | Food products | Capillary electrophoresis | Optimizing the simultaneous separation of these substances | [40] |

Table 5
Experimental matrices for central composite designs: (a) two variables and (b) three variables

| (a) | | | (b) | | | |
|------------------|------------|------------|------------------|------------|------------|------------|
| | x_1 | x_2 | | x_1 | x_2 | x_3 |
| Factorial design | −1 | −1 | Factorial design | −1 | −1 | −1 |
| | 1 | −1 | | 1 | −1 | −1 |
| | −1 | 1 | | −1 | 1 | −1 |
| | 1 | 1 | | 1 | 1 | −1 |
| Axial points | − α | 0 | Axial points | −1 | −1 | 1 |
| | α | 0 | | 1 | −1 | 1 |
| | 0 | − α | | −1 | 1 | 1 |
| | 0 | α | | 1 | 1 | 1 |
| Central point | 0 | 0 | Central point | − α | 0 | 0 |
| | | | | α | 0 | 0 |
| | | | | 0 | − α | 0 |
| | | | | 0 | α | 0 |
| | | | | 0 | 0 | − α |
| | | | | 0 | 0 | α |
| | | | | 0 | 0 | 0 |

Table 6
Some applications of central composite design in analytical chemistry

| Analytes | Samples | Analytical technique | Objective of the study | Ref. |
|---|--------------------------------|--------------------------------|--|------|
| Chlorobenzenes | Environmental water | HPLC | Developing a headspace single-drop micro-extraction procedure using room temperature ionic liquid for determination of trace amounts of these substances | [41] |
| Human immunoglobulin G | Artificial mixture of proteins | Affinity HPLC | Optimizing the purification of these proteins from a mixture | [42] |
| Organochlorine pesticides and polychlorinated biphenyls | Human serum | GC | Developing a procedure for the determination of these substances using headspace solid-phase micro-extraction | [43] |
| Tetracycline, chlortetracycline, oxytetracycline and doxycycline | Pharmaceuticals | Capillary zone electrophoresis | Investigating the influence of the electrolyte composition, pH and concentration, as well as temperature and applied voltage in the separation of the analytes | [44] |
| Volatile compounds | Vinegar | GC | Optimizing the extraction and desorption analytical conditions of a stir bar sorptive extraction for these analytes | [45] |
| Polybrominated diphenyl ethers, polybrominated biphenyls and polychlorinated naphthalenes | Sediment samples | GC–MS | Optimization of the experimental conditions for a method involving microwave-assisted extraction and large-volume injection | [46] |
| Amlodipine, nitrendipine, felodipine, lacidipine and lercanidipine | Human plasma | HPLC | Developing a liquid–liquid extraction method using diethyl ether as organic solvent for determination of five 1,4-dihydropyridines | [47] |
| Nickel | Petroleum | GF AAS | Developing a procedure for the direct determination of Ni using a solid sampling strategy | [48] |
| Aluminum | Juices and soft drink | GF AAS | Developing a preparation method based on ultrasound-assisted pseudo-digestion | [49] |
| Mercury | Gasoline | CV AAS | Optimizing a method for direct aqueous NaBH ₄ reduction of metal in microemulsion medium | [43] |
| As, Cd, Cu, Fe, Mg, Pb and Zn | Mussel tissues | ICP OES | Evaluation of different variables affecting the enzymatic hydrolysis of samples by five enzymes | [50] |
| Hydroxymethylfurfural | Honey | Amperometry | Development of microbiosensors built by photolithographic techniques and based on a Pt microelectrode chip | [51] |

matrices. This design describes a circular domain for two variables, spherical for three variables, and hyperspherical for more than three variables, which accents the uniformity of the studied variables in the experimental domain. Although its matrices are not routable as previous designs, it presents some advantages, such as requiring few experimental points for its application and high efficiency. Other characteristics are presented below:

- (1) requires an experiment number according to $N = k^2 + k + c_p$, where k is the factor number and (c_p) is the replicate number of the central point;
- (2) each variable is studied at a different number of levels, a particularly important characteristic when some variables are subject to restrictions such as cost and/or instrumental constraints or when it is interesting to study a variable at a major or minor number of levels;
- (3) the intervals between its levels present a uniform distribution;
- (4) displacement of the experimental matrix to another experimental region can be achieved using previous adjacent points.

For two variables, the Doehlert design is represented by a central point surrounded by six points from a regular hexagon (Fig. 4). Fig. 4 also shows some possibilities of displacement of the original experimental conditions. For three variables, this design is represented by a geometrical solid called a cuboctahedron [18], and, depending on how this solid is projected in the plane, it can generate some different experimental matrices (Fig. 5). Table 7(a) shows the experimental matrix for two variables, and Table 7(b and c) shows two experimental matrices for three-variable optimization generated by different plane projections of the cuboctahedron.

Applications of the Doehlert design in analytical chemistry are increasing in recent years, mainly because of its advantageous char-

acteristics in relation to other designs. Some examples are shown in Table 8 to illustrate its field of application.

5. Multiple responses optimization in analytical chemistry by using RSM

It is relatively simple to find the optimal conditions for a single response using surface response designs. However, the researcher may be interested in optimizing several responses simultaneously. The simplest strategy to adopt in this case is visual inspection. If the amount of significant factors allows the graphical visualization of adjusted models, and if the numbers of response are

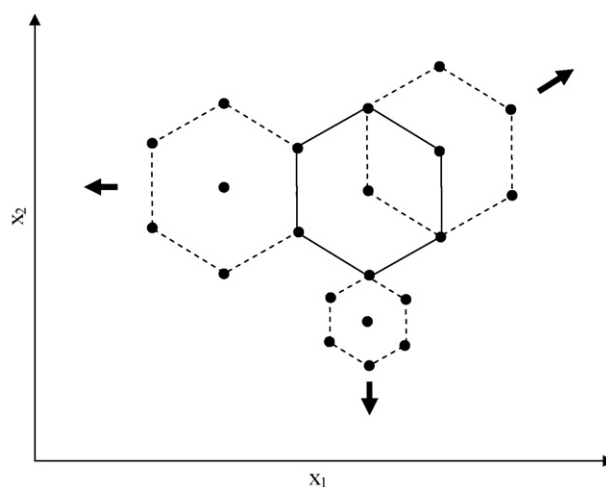


Fig. 4. Doehlert design for the optimization of two variables and some possibilities for the displacement of the initial design using previous points.

Table 7

Doehlert matrices (a) for two variables, (b) three variables for the plane projection "a" of Fig. 5 and (c) three variables for the plane projection "b" of Fig. 5

| (a) | | (b) | | | (c) | | |
|-------|--------|-------|-------|--------|-------|--------|--------|
| x_1 | x_2 | x_1 | x_2 | x_3 | x_1 | x_2 | x_3 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | −1 | 0 | 1 | 0 | 0 |
| 0.5 | 0.866 | 1 | 0 | 0 | 0.5 | 0.866 | 0 |
| −1 | 0 | 0 | 1 | 0 | 0.5 | 0.289 | 0.817 |
| −0.5 | −0.866 | −1 | 0 | 0 | −1 | 0 | 0 |
| 0.5 | −0.866 | −0.5 | −0.5 | 0.707 | −0.5 | −0.866 | 0 |
| −0.5 | 0.866 | 0.5 | −0.5 | 0.707 | −0.5 | −0.289 | −0.817 |
| | | 0.5 | 0.5 | 0.707 | 0.5 | −0.866 | 0 |
| | | −0.5 | 0.5 | 0.707 | 0.5 | −0.289 | −0.817 |
| | | −0.5 | −0.5 | −0.707 | −0.5 | 0.866 | 0 |
| | | 0.5 | −0.5 | −0.707 | 0 | 0.577 | −0.817 |
| | | 0.5 | 0.5 | −0.707 | −0.5 | 0.289 | 0.817 |
| | | −0.5 | 0.5 | −0.707 | 0 | −0.577 | 0.817 |

not very large, the surfaces can be overlapped to enable finding the experimental region that can satisfy all the responses studied [19,10].

If the optimal values for each response are localized in different regions, it will be more difficult to find the conditions that simultaneously satisfy all responses. The level of difficulty increases as these optimum regions become more distant from each other and do not intersect. It is not rare to encounter cases where all surfaces found do not present its optimum under the same set of experimental conditions. Thus, changes in the level of a factor can improve one specific response and have a very negative effect on another.

An approach for solving the problem of the optimization of several responses is the use of a multicriteria methodology. This methodology is applied when various responses have to be considered at the same time and it is necessary to find optimal compromises between the total numbers of responses taken into account. The Derringer function or desirability function [20] is the most important and most currently used multicriteria methodology in the optimization of analytical procedures. This methodology is initially based on constructing a desirability function for each

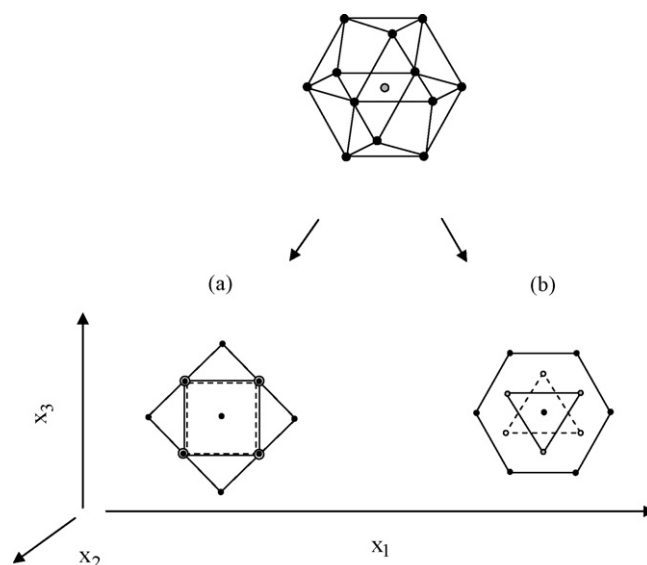


Fig. 5. Doehlert designs for the optimization of three variables originated by the two-plane projection of the cuboctahedron geometric solid.

individual response. In summary, the measured properties related to each response are transformed into a dimensionless individual desirability (d_i) scale. Through the individual functions, the analyst introduces the specifications that each response must fulfill in the measuring procedure. The scale of the individual desirability function ranges between $d = 0$, for a completely undesirable response, and $d = 1$, for a fully desired response, above which further improvements would have no importance. This transformation makes it possible to combine the results obtained for properties measured on different orders of magnitude.

With the individual desirabilities, it is then possible to obtain the overall desirability (D). The overall desirability function D is defined as the weighted geometric average of the individual desirability (d_i)

Table 8

Some applications of Doehlert design in analytical chemistry

| Analytes | Samples | Analytical technique | Objective of the study | Ref. |
|---------------------------|--|--|--|------|
| Cd, Cr, Cu, Mn, Ni and Pb | Saline oil-refinery effluents and vegetables | ICP OES | Optimizing the cloud point extraction of these metals | [52] |
| Cd | Drinking water | F AAS | Optimizing a pre-concentration system that use a mini-column of polyurethane foam loaded with 4-(2-pyridylazo)-resorcinol | [53] |
| Uranium | Natural waters | Molecular absorption spectrometry | Developing a pre-concentration procedure using cloud point extraction | [54] |
| Fe, Zn and Mn | Food | F AAS | Optimizing a procedure for the food samples digestion employing a focused microwave system | [55] |
| Si | Naphta | GF AAS | Developing a method for direct determination of the analyte | [56] |
| Mn | Biological | FI ICP OES | Developing a procedure for pre-concentration of analyte using a column packed with silica gel functionalized | [57] |
| Catechol | Waters | Voltammetry | Optimizing variables associate to the performance of the solid-phase extraction procedure based on molecular imprinting technology | [58] |
| Quinolinic acid | Human plasma | Differential pulse polarography | Developing of a procedure for determining this analyte after solid-phase extraction | [59] |
| Chloroanisoles | Wine | GC | Optimizing the headspace solid-phase micro-extraction | [60] |
| Cholesterol | Milk fat, frozen diet and egg powder | GC | Modeling of the relationship analyte/internal standard to determine cholesterol | [61] |
| Sugars | Food | HPLC | Investigating the derived sugars with <i>p</i> -nitroaniline using microwave irradiation in a pre-column | [62] |
| Herbicide oxidiazin | Water and soil | GC-MS | Optimizing the chromatographic conditions to determine oxidiazin residues | [63] |
| Organochlorine pesticides | Water | GC | Optimizing the solid-phase micro-extraction conditions of polyacrylate-coated fiber | [64] |
| Tropane alkaloids | Belladonna extract | Micellar electrokinetic capillary chromatography | Optimizing the analysis of selected tropane alkaloids | [65] |

according the following equation:

$$D = \sqrt[m]{d_1 d_2 \dots d_m} \quad (16)$$

where m is number of responses studied in the optimization process. Thus, the simultaneous optimization process is reduced to find the levels of factors that demonstrate the maximum overall desirability.

There are several types of transformations possible for obtaining individual desirability. Thus, if the target value (T) for the response y is a maximum, the individual desirability (d) is described by the following equation:

$$d = \begin{cases} 0 & \text{if } y < L \\ \left(\frac{y-L}{T-L}\right)^s & \text{if } L \leq y \leq T \\ 1 & \text{if } y > T \end{cases} \quad (17)$$

where L is the lower acceptable value to the response and s is the weight. Thus, when $s = 1$, the desirability function is linear. When $s > 1$ is chosen, a major importance is given to the points near the target value. When $s < 1$ is chosen, this last demand is of low importance.

However, if the target value for the response y is a minimum, the individual desirability (d) is given by:

$$d = \begin{cases} 1 & \text{if } T < y \\ \left(\frac{U-y}{U-T}\right)^t & \text{if } T \leq y \leq U \\ 0 & \text{if } y > U \end{cases} \quad (18)$$

where U is the upper acceptable value to the response and t is a weight. The same idea for s is applied for t to attribute levels of importance to the target value.

If the target value (T) is located between the lower limit (L) and the upper limit (U), then, a bilateral desirability function must be

used. This function is expressed by the following equation:

$$d = \begin{cases} 0 & \text{if } y < L \\ \left(\frac{y-L}{T-L}\right)^s & \text{if } L \leq y \leq T \\ \left(\frac{U-y}{U-T}\right)^t & \text{if } T \leq y \leq U \\ 0 & \text{if } y > U \end{cases} \quad (19)$$

As demonstrated, t and s control the variation rate of the desirability functions. When these parameters are varied, it is feasible to attribute different desirability to the responses and, consequently, to increase or decrease the range of acceptable values in the optimization process.

The application of desirability functions in analytical chemistry brings advantages as efficiency, economy, and objectivity in the optimization of multiple response procedures. Despite the obvious advantages of this methodology in the optimization of analytical procedures, there are still few applications found in the literature. Derringer functions have been more applied for optimization in chromatographic and related techniques (electrochromatography and electrophoresis) principally because they can establish conditions for the best resolution among several peaks simultaneously. Table 9 shows some applications of the desirability function for the optimization of multiple responses in analytical chemistry.

6. Use of artificial neural networks in RSM

Artificial neural networks (ANNs) offer an attractive possibility for providing non-linear modeling for response surfaces and optimization in analytical chemistry.

ANNs are inspired by the arrangement of cerebral networks and consist of groups of highly interconnected processing elements called neurons. The neurons are arranged in a series of layers: one input layer with neurons representing independent variables, one output layer with neurons representing dependent variables, and several hidden layers that associate the inputs with outputs. Each neuron from one layer is connected with each neuron in the next

Table 9
Some applications of Desirability function in analytical chemistry

| Analytes | Samples | Analytical technique | Objective of the study | Ref. |
|--|--------------------------------|--|--|------|
| Organochlorines and pyrethroids | Tea | GC | Optimizing a method based on matrix solid-phase dispersion and gas chromatography for the determination of multi-residue pesticides | [66] |
| R-Timolol and other impurities | S-Timolol maleate | HPLC | Finding the optimal chromatographic condition for the simultaneous determination of analytes | [67] |
| Anthraquinones and bianthrone | Herbal medicine | Micellar electrokinetic chromatography | Developing a chromatography method for the analysis of anthraquinones and bianthrone in rhubarb crude drugs | [68] |
| Organomercury compounds and Hg(II) | Seawater | GC–MIP OES | Developing a method for determination of these species | [69] |
| Methyl <i>tert</i> -butyl ether, <i>tert</i> -butyl alcohol, benzene, toluene, ethylbenzene and xylene isomers | Groundwater | GC–MS | Developing a method for the simultaneous determination of these substances | [70] |
| Methylmercury and Hg(II) | Biological | GC | Developing a method for the extraction based microwave-assisted extraction and solid-phase micro-extraction | [71] |
| Methylphenobarbital enantiomers and phenobarbital | Human plasma | HPLC | Developing an automated liquid chromatographic method for the simultaneous determination of analytes | [72] |
| Local anesthetics | Human plasma | HPLC | Developing an automated method involving dialysis, clean-up and enrichment of the dialysate on a pre-column packed with a strong cation-exchange phase | [73] |
| Vitamins B ₆ and B ₁₂ , dexamethasone and lidocaine | Pharmaceutical preparations | Capillary electrophoresis | Developing a method for the simultaneous determination of these four substances in pharmaceutical preparations | [74] |
| Cu, Bi and Li | Tap-water and synthetic alloys | Adsorptive stripping voltammetry | Developing a method for simultaneous determination of these metals | [75] |

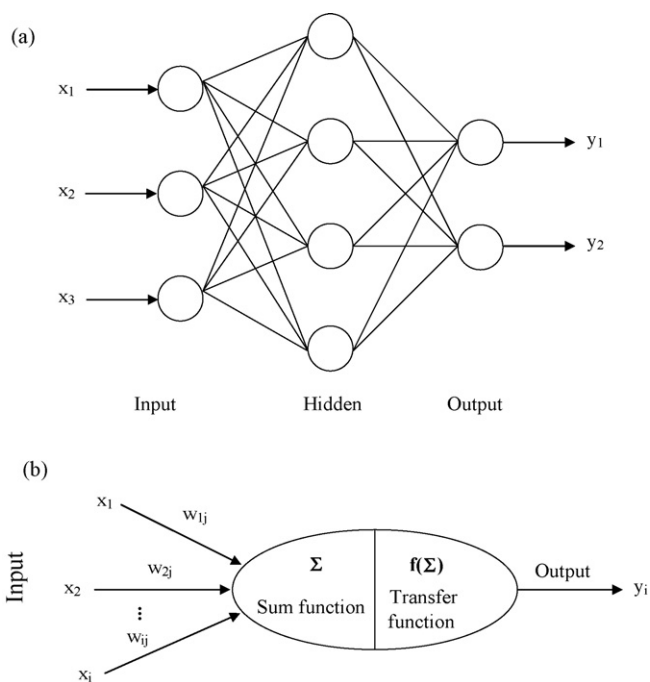


Fig. 6. Artificial neural network: (a) scheme of a three-layer network; (b) operation of a single neuron.

layer. The pattern of interconnection among the neurons is called the network “architecture”, and it can be conveniently represented on a graph (Fig. 6(a)). Data generated from the experimental design can be used as relevant inputs, as well as outputs, for ANN training [21–23].

The training is carried out by adjusting the strength of connections between neurons with the aim to adapt the outputs of the entire network to be closer to the desired outputs or to minimize the sum of the training data. During the training phase, each neuron receives the input signals x_i from n neurons, aggregates them by using the weights (w_{ij}) of the synapses, and passes the result after suitable transformation as the output signal y_i (Fig. 6(b)) as a

function of the sum, according to Eq. (20):

$$y_i = f \left(\sum_{j=1}^n x_j w_{ij} \right) \quad (20)$$

where f is the transfer function that is necessary to transform the weighted sum of all the signals connecting with a neuron. The most widely used transfer function is presented in Eq. (21):

$$f = \frac{1}{1 + e^{-cx}} \quad (21)$$

where c is a constant that determines the slope of the sigmoid function.

The training phase is finished when the square error is minimized across all training experiments. Once ANN has been trained, it has a good predictive capability and ability to accurately describe the response surface even without any knowledge about the physical and chemical background of the modeled system [14,22].

ANN offers an alternative to the polynomial regression method as a modeling tool. Classical RSM requires the specification of a polynomial function such as linear, first-order interaction, or second-order quadratic, to be regressed. Moreover, the number of terms in the polynomial is limited to the number of experimental design points, and the selection of the appropriate polynomial equation can be extremely cumbersome because each response requires its own individual polynomial equation.

The ANN methodology provides the modeling of complex relationships, especially non-linear ones, that may be investigated without complicated equations. ANN analysis is quite flexible in regards to the number and form of the experimental data, which makes it possible to use more informal experimental designs than with statistical approaches. Also, neural network models might have better predictive power than regression models. Regression analyses are dependent on predetermined statistical significance levels, and less significant terms are usually not included in the model. With the ANN method, all data are used, potentially making the models more accurate [82].

Using ANN modeling for the optimization of analytical methods has been applied mainly for the development of chromatographic methods. Table 10 shows some applications of artificial neural network modeling in analytical chemistry.

Table 10
Some applications of RSM combined with artificial neural networks in analytical chemistry

| Analytes | Samples | Analytical technique | Objective of the study | Ref. |
|--|--|--------------------------------|---|------|
| Fosinopril sodium and its degradation product fosinoprilat | Pharmaceuticals | GC–MS | Combining central composite design and ANNs in optimization of mobile phase composition for analysis | [76] |
| Herbicides | Waters | HPLC | Optimizing of the linear gradient separation of 10 herbicides consisting of a mixture of acids, bases and neutrals employing a single ANN for modeling the response surface | [77] |
| Neuroprotective peptides | Mixture of peptides | HPLC | Using experimental design conjunction with artificial neural networks for optimization of isocratic ion-pair separation of neuroprotective peptides | [22] |
| Huperzine A | Pharmaceutical products and biological liquids | Capillary electrophoresis | Using of the experimental design combined with the artificial neural networks for optimization of the drug separation | [78] |
| <i>cis</i> - and <i>trans</i> -resveratrol | Australians wine | Capillary zone electrophoresis | Optimizing the solid-phase extraction employing central composition design and ANN | [79] |
| Triazine herbicides | Waters | HPLC | Prediction of retention factors of the studied herbicides | [80] |
| Antimicrobial agents | Cosmetics | HPLC | Using of experimental design/ANN to correlate the retention time of each analyte (20 typical antimicrobial substances) to the variables and their interactions | [81] |
| Ruthenium | Refined ore | Spectrophotometry | Coupling experimental design and ANN for the optimization of an on-line microwave flow injection system | [82] |
| Hydrochlorothiazide and amiloride | Pharmaceuticals | HPLC | Comparing artificial neural networks for response surface modeling in HPLC with multiple regression methods | [83] |

7. Conclusions

Application of response surface methodology in the optimization of analytical procedures is today largely diffused and consolidated principally because of its advantages to classical one-variable-at-a-time optimization, such as the generation of large amounts of information from a small number of experiments and the possibility of evaluating the interaction effect between the variables on the response.

In order to employ this methodology in experimental optimization, it is necessary to choose an experimental design, to fit an adequate mathematical function, and to evaluate the quality of the fitted model and its accuracy to make previsions in relation to the experimental data obtained.

The central composite design is still the symmetrical second-order experimental design most utilized for the development of analytical procedures. The application of three-level factorial designs is not frequent, and the use of this design has been limited to the optimization of two variables because its efficiency is very low for higher numbers of variables. However, the Box–Behnken and Doehlert designs present more efficient matrices and have increased the number of published works in recent years.

Multiple response optimization using desirability functions has until now had its utilization limited to the chromatographic field, its related techniques, and to electrochemical methods. However, its principles can be applied to the development of procedures using various analytical techniques, which demand a search for optimal conditions for a set of responses simultaneously.

Finally, as an alternative to classical modeling, an adaptive learning technique that utilizes neural networks combined with experimental design, can be employed to model a dependence relation. This approach has demonstrated a superior accuracy in data learning and prediction over the traditional RSM.

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