



Review article

Simplex optimization: A tutorial approach and recent applications in analytical chemistry



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ABSTRACT

Simplex is an optimization technique that, in a general sense, does not require the use of complex mathematical and statistical tools and can be very useful in the development of analytical methods. In this work, a tutorial reviewing the basic concepts, fundamental approach and a work guide are presented for those who contemplate simplex optimization as a tool to develop analytical methods. The characteristics and rules for applying the basic and modified simplex algorithms commonly used for optimization are also described. Some studies demonstrating the application of simplex optimization in analytical chemistry are discussed to illustrate the applicability of this technique.

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1. Introduction

An optimization process aims to improve the performance of a system, a process, a procedure or a product, so that the greatest benefits can be achieved. In analytical methods, several variables (also called

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factors) can influence the magnitude of the studied response. Thus, when analytical methods are developed, they also require optimization through the investigation of variables and their respective levels [1,2].

The term optimization has traditionally been used in analytical chemistry to designate a set of experiments used to find the proper conditions for carrying out a method, in order to achieve the best possible responses, ensuring the best analytical characteristics. Moreover, it is necessary to determine whether the optimum conditions are those that result in better accuracy, higher sensitivity, lower quantification limits, the largest number of measures per time units and lower costs. Notwithstanding that, the costs of the analytical procedure should not be ignored and it is generally assumed that the best accuracy and the highest sensitivity are the most significant factors to be considered [3].

Optimization processes have been traditionally performed by monitoring the influence of one variable at a time on a given experimental response. In this type of optimization, called univariate optimization, when the level of a factor is changed, the levels of other factors of interest are kept at constant values. As a result, this technique does not allow assessing the effects of interactions between variables. In recent years, multivariate chemometric tools have been frequently applied to the optimization of analytical methods; therefore, advantages such as the reduction in the number of experiments are taken into account, hence resulting in lower costs of reagents and time consumption. There are, thus, a large number of chemometric tools, such as two-level factorial designs, mixture designs, Doehlert and Box–Behnken designs, etc., which allow multivariate optimization [4].

The simplex method suggests the optimization of various studied factors without the need to use more specific mathematical-statistical expertise as required in response surface methodology [5]. The original simplex method (basic algorithm) was developed in 1962 [6]. The first application of the simplex method in analytical chemistry occurred in 1969 [7] for the optimization of a colorimetric method in the determination of sulfur; since then, the simplex method has been satisfactorily applied to the development of analytical procedures in the determination of several analytes of interest.

This paper covers a tutorial approach about simplex optimization and its application in analytical chemistry. The initial part of the work reviews the theoretical principles and presents a work guide for those who intend to use this optimization methodology. The second part presents some examples from the literature to illustrate the use of simplex optimization in the development of analytical methods to determine various substances in different matrices.

2. Brief introduction to the simplex methodology

Simplex optimization is performed by the displacement of a geometric figure with $k + 1$ vertexes in an experimental field toward an optimal region, where k equals the number of variables in a k -dimensional domain. Simplex in one dimension is therefore represented by a line, while in two dimensions it is represented by a triangle, in three dimensions, by a tetrahedron, and hyperpolyhedrons represent multiple dimensions [8,9].

2.1. Principles of the simplex method

The principle of the simplex method is the displacement of an initial design through the experimental region studied in order to avoid regions with undesirable responses. Thus, the simplex displacement is carried out by the reflection of the experimental point showing the worst response generating a new simplex that should be once again analyzed and displaced to the optimal region. Fig. 1(a) illustrates this process in regard to the optimization of two variables: x_1 and x_2 . Fig. 1(b) shows the simplex optimization regarding three variables through the displacement of the initial simplex represented by the geometric solid (tetrahedron) [10].

Sequential optimization methods such as the simplex are applied by performing few experiments at a time. The results obtained in each stage are used to support decisions in regard to experiments to be conducted later along the process that aims to reach the region of optimum response. Essentially, sequential methods comprehend simple and fast processes because they do not require mathematical functions to establish relation between factors and responses during the optimization process [11].

2.2. Characteristics of the simplex method

The simplex method is originally sequential, i.e., the next stage must only be conducted after evaluating the previous response, thereby making the method more suitable for the optimization of fast response systems. One limitation is the impossibility of working with qualitative variables. Yet, depending on the characteristics of the systems to be optimized, simplex methods are chosen because they are simple and fast. Simplex optimization is perhaps the one that best suits the most common situations encountered in the laboratory. Even though its application does not provide detailed information concerning the variables under study, it is practical and fast and allows finding the optimal region with a good safety margin [12].

Simplex optimization does not require the use of statistical tests for assessing the significance (t and F , for example) due to the following reasons: (1) if the differences in responses are large when compared with the experimental error, the simplex will move in the right direction and (2) in case the differences are small, so that they undergo influence of experimental error, the simplex will move in the wrong direction; yet, this would lead to an undesirable response and the direction would immediately be corrected by the movement rules.

2.3. Classifications of the simplex method

Some algorithms were developed for the simplex displacement along the experimental region. The original basic algorithm is the simplest and easiest to implement because it relies only on the reflection movement of the point corresponding to the worst response. The modified algorithm allows a greater range of motions and, therefore, the most accurate and efficient location for the optimum conditions. There is also the super-modified algorithm in which shape and size can be better adjusted according to the topology and the characteristics of the experimental region, thereby making the search for optimal conditions even more efficient. However, this algorithm requires the fitting of polynomial functions and that makes the mathematical treatment more complex [13,14]. The latter algorithm will not be addressed in this study.

2.4. Available software for simplex optimization

Various software packages can be used to help researchers in the application of simplex optimization. The basic simplex implicates few decision making opportunities and its movements can be easily accomplished with the help of a common electronic spreadsheet such as the Microsoft Excel®. However, the modified simplex requires the use of more specific software such as the Multisimplex® [15]. There are also free software packages for simplex optimization as the one available on the Chemkeys website [16].

3. Basic simplex

The fixed-size simplex, or basic simplex, consists of a regular geometric figure that does not vary in size during the displacement process for the optimum conditions. This characteristic of the basic simplex makes choosing the size of the initial simplex a crucial step for the efficiency of the optimization process. Thus, the experience of the

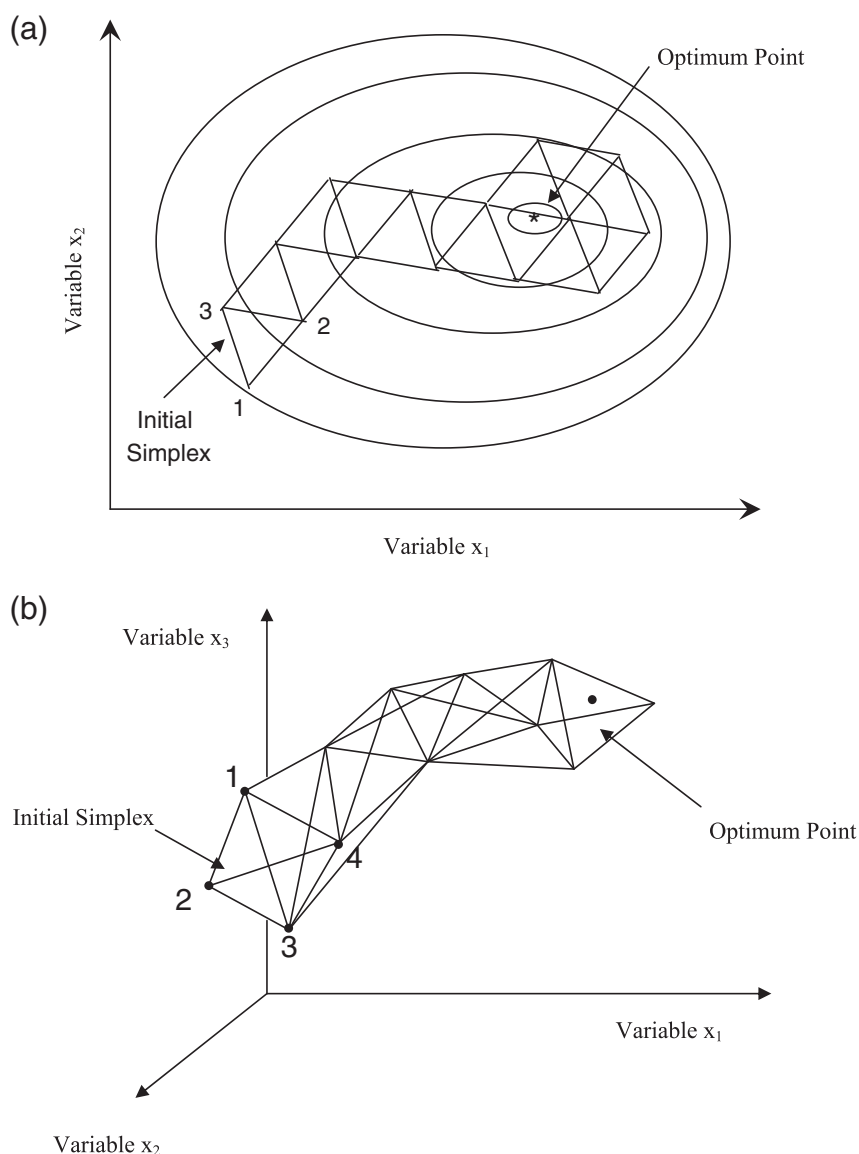


Fig. 1. Examples of the displacement of initial simplexes to the region of optimum response (a) optimization of two variables and (b) optimization of three variables.

researcher concerning the system to be studied is very important in making decisions about the size of the simplex to be initially used to optimize certain experimental procedures.

3.1. Rules for the application of a basic simplex

The following rules explain how to apply the basic simplex method in the optimization of a system for k variables: (1) Set the number of k variables that will lead to an initial simplex with $k + 1$ experimental points or vertices. For example, for $k = 2$, there are three vertices that can form a triangle when joining themselves; (2) generate the initial simplex (coded levels presented in Table 1 can be used for this purpose); (3) carry out the experiments under the experimental conditions and make the reflection of the vertex presenting the worst response, following Section 3.2; (4) a new simplex is obtained with this new point, hence discarding the vertex that had the worst response and utilizing the previous vertices. The responses must again be evaluated and the previous process must be repeated until the optimal region is found; (5) If the new vertex obtained by reflection shows the worst response of the new simplex, it becomes necessary to reject the second worst vertex of the previous simplex in order to avoid oscillation between the two simplexes, which will not leave the experimental region.

3.2. Calculation of new vertices in a basic simplex

Suppose W is the vertex that provides the worst response, B the best response and N (next to better) the second-worst response. In order to find an expression for the calculation of the coordinates of the new R vertex obtained from a reflection, see Fig. 2; the reflection of the worst

Table 1
Multiplication factors for the calculation of the vertexes of an initial simplex.

Vertex	Variables								
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9
1	0								
2	1	0							
3	0.50	0.87	0						
4	0.50	0.29	0.82	0					
5	0.50	0.29	0.20	0.79	0				
6	0.50	0.29	0.20	0.16	0.78	0			
7	0.50	0.29	0.20	0.16	0.13	0.76	0		
8	0.50	0.29	0.20	0.16	0.13	0.11	0.76	0	
9	0.50	0.29	0.20	0.16	0.13	0.11	0.094	0.75	0
10	0.50	0.29	0.20	0.16	0.13	0.11	0.094	0.083	0.75

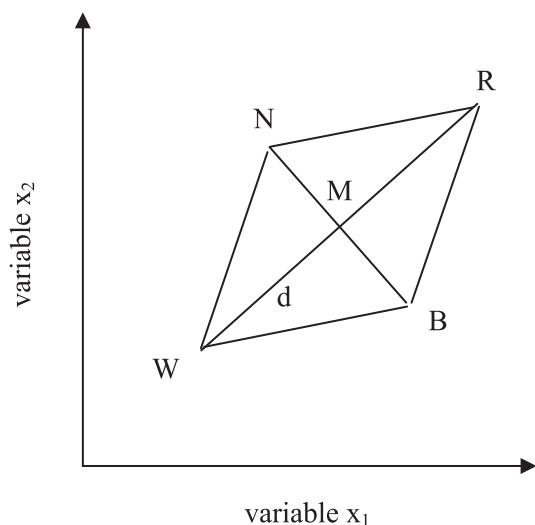


Fig. 2. Calculation of the coordinates of the new vertex, which was obtained by reflecting the point with the worst response.

point of the *BNW* simplex is represented, whereas the *R* point that defines the new *BNR* simplex in the optimization of two variables is obtained. It is noted that the *R* vertex is equally far from the *M* midpoint on the *NB*-side, as well as the *W* vertex. Thus, finding the coordinates of the new *R* vertex merely requires adding the *d* distance value to this midpoint.

$$R = M + d \quad (1)$$

The *d* distance value is calculated as follows:

$$d = M - W \quad (2)$$

By replacing the value of *d* in the previous expression we obtain:

$$R = M + (M - W) \Rightarrow R = 2M - W \quad (3)$$

The *M* midpoint value regarding any number of variables studied is provided by the expression:

$$M = \frac{1}{N} \sum_{j=1}^N v_j \quad (4)$$

where *N* stands for the number of variables, *i* represents the vertex index of the worst response, which shall be eliminated, and *j* comprehends the considered vertex.

3.3. An example of a basic simplex application in analytical chemistry

As an example, an inedited work will be presented, developed in our laboratory, in which a basic simplex algorithm has been used to optimize an on-line preconcentration system for the determination of nickel ions in samples of vegetable leaves by flame atomic absorption spectrometry (FAAS). This preconcentration system is based on solid phase extraction of nickel in a minicolumn filled with Amberlite XAD-4 (polystyrene-divinylbenzene polymer) resin impregnated with 5-Br-PADAP (2-(5-bromo-2-pyridylazo) 5-diethylaminofenol), metal elution with a solution of hydrochloric acid, and reading by FAAS. The variables that most affect the performance of the pre-concentration (pH and sample flow rate) were optimized using a basic simplex.

For the definition of the simplex, the variables that will be subject to optimization must be determined (in this case, pH and sample flow rate). Therefore, the initial value and the dimension of the variation

step of each simplex variable are chosen. Alternatively, Table 1 can be used in the construction of the initial simplex with up to 9 (nine) factors.

With regard to the pH variable, the value of 5.00 was attributed as initial value, while a sample flow rate of 2.4 mL min^{−1} was chosen. As a variation step, 1.0 and 1.2 mL min^{−1} were attributed to pH and sample flow rate, respectively. Calculations of the initial values of the vertexes are presented in Table 2.

To calculate the new vertex, the vertex with the worst response (vertex 1, whose absorbance signal is 0.022) is excluded and the average of the two remaining vertexes is calculated according to Eq. 5:

$$M = \frac{1}{2} \left(\begin{bmatrix} 3.6 \\ 5.00 \end{bmatrix} + \begin{bmatrix} 3.0 \\ 5.87 \end{bmatrix} \right) = \begin{bmatrix} 3.3 \\ 5.44 \end{bmatrix} \quad (5)$$

The reflected point should be calculated using Eq. 6:

$$R = 2 \begin{bmatrix} 3.3 \\ 5.44 \end{bmatrix} - \begin{bmatrix} 2.4 \\ 5.00 \end{bmatrix} = \begin{bmatrix} 4.2 \\ 5.88 \end{bmatrix} \quad (6)$$

Thus, the coordinates of the new vertex are attainable—their pH and flow rate sample values are 4.2 mL min^{−1} and 5.88, respectively. A new trial must be performed under these conditions in order to obtain the corresponding response. The three responses are once again analyzed by reflecting the worst vertex until the optimum conditions for the system in question are reached.

The experiments carried out by application of the basic algorithm from the initial to the last simplexes are shown in Table 3.

At the end of the optimization process, the following optimal values were found for the variables: pH = 8.54 and sample flow rate = 6.0 mL min^{−1} (values corresponding to the vertex 10 in the absorbance *X* vertex graphic, as seen in Fig. 3).

4. Modified simplex

In the year 1965 [17], Nelder and Mead proposed some changes in the basic algorithm; such changes were intended to allow the execution of additional movements that are more suitable to the location of the optimum point – with sufficient accuracy and clarity – thus allowing a faster simplex development. This is possible because the initial size of the figure in this algorithm can be constantly changed by expansion and contraction of the reflected vertices. Thus, the following major moves are practicable for the modified algorithm: reflection (*R*), expansion (*E*), contraction (*C_R*) and contraction with change of direction (*C_w*). Fig. 4 shows these types of movements, beginning with a *BNW* simplex for two variables. Thus, *BNR* is the simplex obtained by reflecting the vertex with the worst response of the initial simplex, while *BNE* is the simplex obtained by expansion; *BNC_w* is the simplex obtained by the contraction movement toward the vertex with the worst response; finally, *BNC_R* is the simplex obtained by the contraction movement from the *R* vertex.

4.1. Rules for a modified simplex application

As in the basic algorithm, the first movement of the modified simplex should be a reflection. The response obtained for the reflected *R*

Table 2

Calculation of the initial vertices of the simplex optimization of the online pre-concentration system.

Vertex of initial simplex	Sample flow rate	pH
1	2.4 + (VS _{SF} , 0) = 2.4	5.00
2	2.4 + (VS _{SF} , 1) = 3.6	5.0 + (VS _{pH} , 0) = 5.00
3	2.4 + (VS _{SF} , 0.50) = 3.0	5.0 + (VS _{pH} , 0.87) = 5.87

VS: variation step; SF: sampling flow rate.

Table 3

Values obtained from simplex optimization.

Experiment	Vertex	Sample flow rate (mL min ⁻¹)	pH	Analytic signal (Abs)
01	I	2.4	5.00	0.022
02	I	3.6	5.00	0.031
03	I	3.0	5.87	0.038
04	R	4.2	5.88	0.044
05	R	3.6	6.76	0.058
06	R	4.8	6.77	0.065
07	R	4.2	7.66	0.087
08	R	5.4	7.68	0.096
09	R	4.8	8.51	0.106
10	R	6.0	8.54	0.182
11	R	5.4	9.38	0.118
12	R	6.6	9.41	0.099

I = initial simplex design, R = reflection of the vertex with the worst response.

vertex (note that R is obtained with $\alpha = 1$) should be evaluated and can be comprehended in the following situations:

- When R is better than B, it indicates that the simplex may be moving to the desired region. The simplex should therefore be expanded for the hypothesis to be verified;
 - If $E > R$, the hypothesis is correct and we should work with the BNE simplex;
 - If $E < R$, the BNR simplex should be maintained.
- When $N < R < B$, the BNR simplex should be maintained.
- When $R < N$, it indicates that the size of the simplex is too large, in that case, it becomes necessary to perform a contraction.
 - If $W < R < N$, it means that the size of simplex is wrong, but the direction is correct; a contraction is therefore made and the new simplex is going to be the BNC_R ;
 - If $R < W$, it means that the size and the direction of the simplex are both wrong; therefore, a contraction with change of direction must be made and the new simplex is going to be the BNC_W .

4.2. Calculation of new vertices in a modified simplex

Calculations for the vertexes generated by the movement of the simplex using the modified algorithm can be synthesized by the Eq. 7:

$$NV = M + \alpha(M - W) \quad (7)$$

where NV represents the new vertex, M stands for the average of the best vertexes, α is the movement coefficient of the simplex, and W

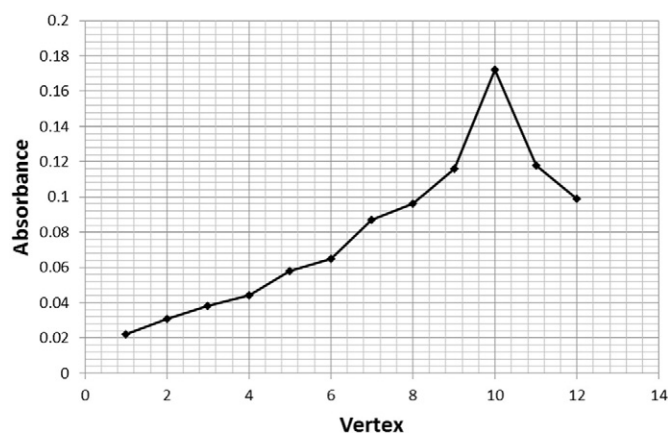


Fig. 3. Possible movements for a modified simplex exemplified for the optimization of two variables.

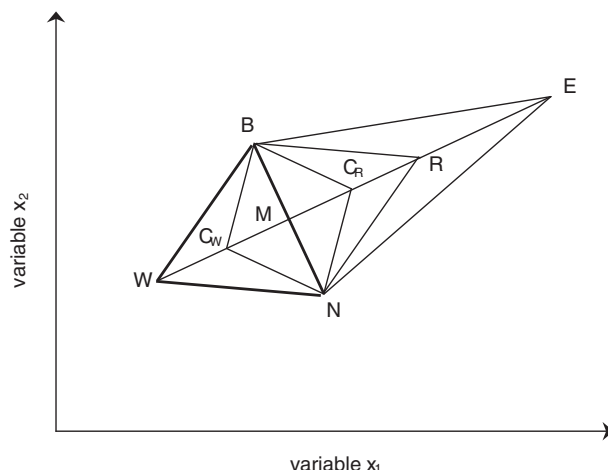


Fig. 4. Evaluation of the analytical signal in relation to the vertices defined by the basic algorithm of the simplex method.

represents the vertex with the worst response. NV may correspond to the R, E, C_R and C_W vertexes. The more commonly adopted α values in the movement of modified simplex (along with the formulas for each type of vertex) are presented in Table 4; other values can also be used.

4.3. An example of a modified simplex application in analytical chemistry

As an example, a work developed by Srijaranai et al will be reviewed [18]. In this study, a modified simplex has been used for the purpose of optimizing the analysis of metal chelates of 4-(2-pyridylazo) resorcinol (PAR) with cobalt, nickel and chromium, utilizing ion pairing reversed phase high performance liquid chromatography (IP-RP-HPLC).

The method is based on the formation of metal complexes in a pre-column. The complexes are kinetically stable during the chromatographic process. The main objective of optimization was to establish the mobile phase composition. The optimized variables were: percentage of modifier (acetonitrile) concentration of acetate buffer and the concentration of the ion-pairing agent (long chain alkyl ions with charge opposite to the analyte charge). The pairing agent utilized was the tetrabutylammonium bromide (TBABr). Chromatographic response function (CRF) was used as a quality criterion. This function combines several parameters such as the resolution between adjacent peaks, the number of detected peaks, and the time of analysis, among others.

The experiments described by the initial simplex were carried out and the calculation of their CRF values is shown in Table 5. The simplex was moved toward the direction determined by the rules of the modified algorithm presented in Section 4.1, hence performing the movements of reflection, expansion, contraction and contraction with change of direction (intended to maximize the response: the chromatographic response function, CRF).

As an example, the vertex 5 is found by the reflection of the vertex that showed the lowest response among the four vertices of the initial

Table 4Values for α normally adopted for the movement of a modified simplex and formulas for calculating the new vertices.

Movement	Vertex symbol	α value	Formula
Reflection	R	1	$R = 2M - W$
Expansion	E	2	$E = 3M - 2W$
Contraction	C_R	0.5	$C_R = 1.5M - 0.5W$
CCD	C_W	-0.5	$C_W = 0.5M + 0.5W$

CCD = contraction with change of direction, M = mean of the best vertex, W = worst vertex.

Table 5

Values for the vertexes obtained by the development of a modified simplex.

Vertex	Movement	Acetonitrile (%)	Buffer ^a	TBABr ^a	CRF
1	I	30.0	3.0	1.0	25.54
2	I	33.0	3.5	1.2	23.00
3	I	31.0	5.0	1.2	23.60
4	I	31.0	3.5	2.0	27.20
5	R	28.4	4.1	1.6	30.25
6	E	26.1	4.4	1.8	26.49
7	R	28.6	2.0	1.8	29.29
8	R	29.0	3.4	2.6	32.13
9	E	28.5	3.6	3.4	34.41
10	R	26.2	2.9	2.6	29.24
11	C _R	27.4	3.1	2.5	26.91
12	R	27.2	5.2	3.2	26.39
13	C _w	28.6	2.8	2.2	30.52
14	R	29.6	3.9	2.3	28.32
15	C _R	29.1	3.7	2.4	29.82
16	R	29.0	2.7	3.8	33.50
17	R	28.3	2.3	3.8	33.88
18	R	28.6	3.0	5.2	34.25
19	R	29.1	3.9	4.4	33.19

I = vertex of initial simplex; R = vertex originated by a reflection, E = vertex originated by an expansion; C_R = vertex originated by a contraction in the reflected vertex; C_w = vertex originated by a contraction considering the worst response.

^aConcentration in mmol/L.

simplex (vertex 2). Thus, this vertex is ignored and the mean of the coordinates of the vertices with the best responses is found, as shown in Eq. 8:

$$M = \frac{1}{3} \left(\begin{vmatrix} 30.0 \\ 3.0 \\ 1.0 \end{vmatrix} + \begin{vmatrix} 31.0 \\ 5.0 \\ 1.2 \end{vmatrix} + \begin{vmatrix} 31.0 \\ 3.5 \\ 2.0 \end{vmatrix} \right) \Rightarrow M = \begin{vmatrix} 30.7 \\ 3.8 \\ 1.4 \end{vmatrix} \quad (8)$$

Reflection is performed using the coordinates of the midpoint and the vertex with the worst response.

As $R = 2M - W$, we have:

$$R = 2 \begin{vmatrix} 30.7 \\ 3.8 \\ 1.4 \end{vmatrix} - \begin{vmatrix} 33.0 \\ 3.5 \\ 1.2 \end{vmatrix} \Rightarrow R = \begin{vmatrix} 28.4 \\ 4.1 \\ 1.6 \end{vmatrix} \quad (9)$$

By performing the experiment in vertex 5, a better response was found than the best response found in the initial simplex. Thus, according to rule "a" of Section 4.1, an expansion should be made. The vertex corresponding to the expansion is calculated as follows:

For $E = 3M - 2W$:

$$E = 3 \begin{vmatrix} 30.7 \\ 3.8 \\ 1.4 \end{vmatrix} - 2 \begin{vmatrix} 33.0 \\ 3.5 \\ 1.2 \end{vmatrix} \Rightarrow E = \begin{vmatrix} 26.1 \\ 4.4 \\ 1.8 \end{vmatrix} \quad (10)$$

Yet, it is noted that the vertex corresponding to the expansion had a response inferior to that of the previous vertex (26.49 for 30.25). It is therefore necessary to follow the rule "a2" and return to the previous simplex.

Another example implies that the vertex 10 was obtained by reflecting the worst vertex (vertex 4) for the vertices with the best responses (5, 7 and 9). However, when analyzing the response of the new vertex in relation to the others, it is verified that the rule "c1" should be employed; the application of such rule is owing to the fact that the observed response (29.24) was better than the worst response

(27.20), but worse than the second best response (29.29). In other words, the simplex must be submitted to a contraction. Vertex 11 is found by the movement of contraction in relation to the previous vertex.

$$M = \frac{1}{3} \left(\begin{vmatrix} 28.4 \\ 4.1 \\ 1.6 \end{vmatrix} + \begin{vmatrix} 28.6 \\ 2.0 \\ 1.8 \end{vmatrix} + \begin{vmatrix} 28.5 \\ 3.6 \\ 3.4 \end{vmatrix} \right) \Rightarrow M = \begin{vmatrix} 28.5 \\ 3.23 \\ 2.27 \end{vmatrix} \quad (11)$$

For $C_R = 1.5M - 0.5W$:

$$C_R = 1.5 \begin{vmatrix} 28.5 \\ 3.23 \\ 2.27 \end{vmatrix} - 0.5 \begin{vmatrix} 31.0 \\ 3.5 \\ 2.0 \end{vmatrix} \Rightarrow C_R = \begin{vmatrix} 27.3 \\ 3.1 \\ 2.4 \end{vmatrix} \quad (12)$$

As what was done in the example from Section 3.3, the experiments were conducted using the vertices set up to the experimental conditions in which high values were observed in the CRF. Notwithstanding the fact that the highest CRF has been obtained in vertex 9, the authors have observed that the experimental conditions represented by the values of variables regarding the vertexes between 16 and 19 were considered to be more robust. Thus, the conditions of the experiment 18 were classified as optimal to perform the analysis.

Fig. 5a–c shows the values of the studied parameters as a function of the vertex number. The percentage of acetonitrile showed little variation and a trend for a CRF close to 29. On the other hand, the buffer concentration showed considerable variation and no obvious tendency. The change in the concentration of TBABr showed a clear tendency to reach an optimal region, close to a CRF value between 4.5 and 5.

The lack of sensitivity of CRF to the concentration of acetonitrile (Fig. 5a) concerning the experimental limits studied suggests that the organic modifier is not a critical factor in the chromatographic behavior of the three metallic complexes in question. The implications for the concentration of the organic modifier in the levels studied are then perceived as appropriate.

With regard to the buffer concentration (Fig. 5b), the authors have reported that the pH is practically maintained constant, thereby seeming that the buffer concentration (in the range investigated during the optimization process) is not so important. This behavior is already expected for the acid–base complexes of Lewis, in which the binder needs to be well ionized (high pH), but the pH should be low in order to prevent the formation of metal hydroxide (lower pH).

The clear dependence of CRF in relation to the concentration of TBABr (Fig. 5c) suggests that metallic complexes and their retention in the reversed phase column strongly depend on a series of equilibria of the ionic pair between the mobile and the stationary phases.

5. Some applications of simplex optimization in analytical chemistry

Besides the several benefits already presented about the simplex methods, their use in analytical chemistry is reported in the literature in a little more than a hundred of papers. In analytical chemistry, the simplex method has been applied in the optimization of instrumental parameters, such as in ICP OES, for example [19]. Santos et al. [23] emphasized that the characteristics of the simplex method are quite proper for the optimization of automated analytical systems for the reason that the levels of the parameters can easily be varied. In addition, these kinds of systems can generate rapid responses—a demand of the simplex method. The simplex optimization (with basic, modified, or super modified algorithm) has been used in analytical chemistry for the optimization of both the operating parameters of the equipment and the variables related to procedures using various techniques and analytical methods (liquid and gas chromatography, molecular and atomic spectrometry, etc.) to improve the determination of substances of environmental, nutritional, biological and pharmacological interests.

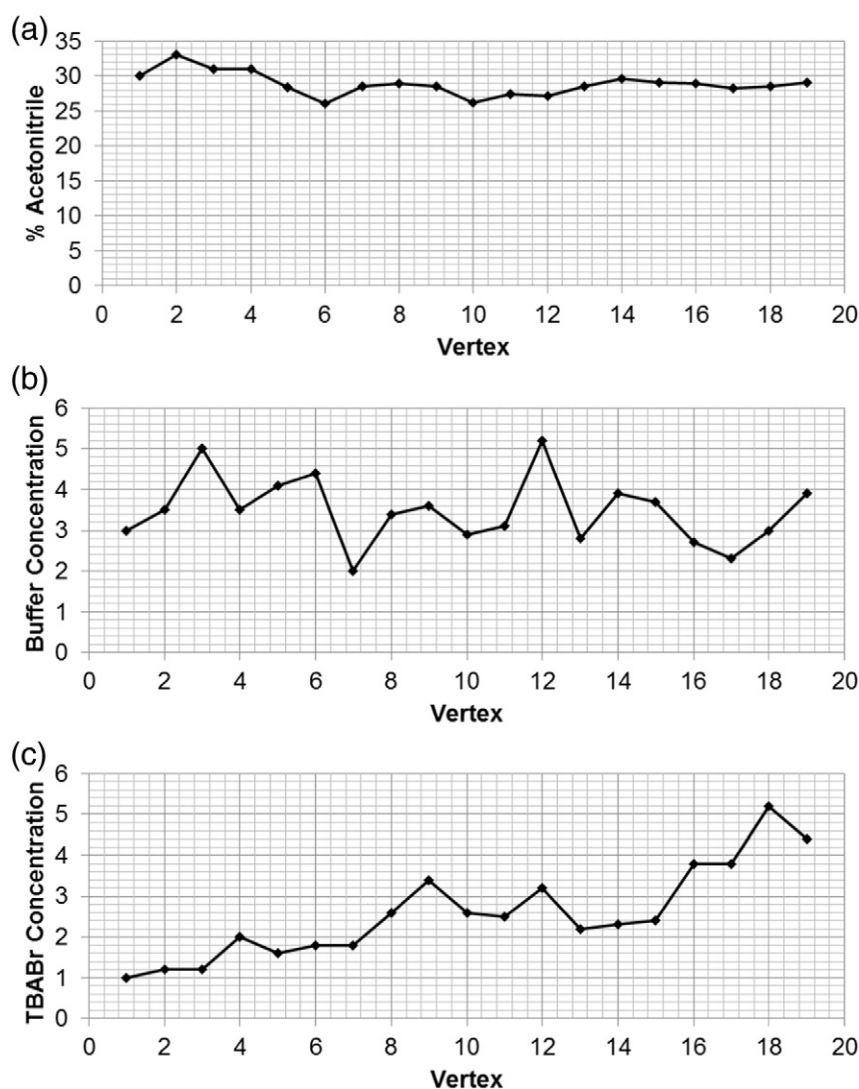


Fig. 5. Analysis of the values of variables concerning each variable, according to the vertex defined by the simplex method, (a) % acetonitrile, (b) concentration of acetate buffer and (c) TBABr concentration.

Only few studies that employ the basic algorithm can be found. Perhaps this is due to their disadvantages in relation to the modified algorithm, such as the need to determine the size of the original simplex risking not succeeding in the accurate location of the optimal point.

Santos et al. [23] developed an automated flow injection method to determine trace amounts of manganese from vegetal leaves and river waters using flame atomic absorption spectrometry (FAAS) after online preconcentration of this metal using an Amberlite XAD-4 minicolumn modified with 2-aminothiophenol. Variables associated with the preconcentration system performance, such as pH, sampling flow rate and eluent concentration were optimized using a basic simplex. This automated method was applied to determine manganese in river waters and vegetal leaves (chive, spinach, cauliflower and cabbage).

Pasamontes and Callao [28] have used a basic simplex to find optimal values in an analytical procedure that allows simultaneous determination of two substances with very similar physical and chemical characteristics (amoxicillin and clavulanic acid) using sequential injection analysis (SIA) coupled with a diode-array spectrophotometric detector and multivariate curve resolution with alternating least squares. To allow simultaneous optimization of these two substances, a multi-response optimization based on desirability function was applied. In this approach, each experimental response was transformed into desirability values. The authors emphasize that it is important to establish an

appropriate size for the initial simplex when working with the basic algorithm, regardless of the adopted response.

Simplex optimization using the basic algorithm was used by Ensafi and Chamjangali [35] to find the best analytical characteristics to determine periodate and iodate in water using a flow injection method. The method was based on the reaction of these analytes with pyrogallol red in sulfuric acid media and the reaction was monitored by measuring the decrease in absorbance of pyrogallol red at 470 nm.

An analytic method for the determination of phenoxy acid herbicides and 2,4-D esters in soil samples by GC-FID was also developed by Santos-Delgado et al. [39] Four variables (methylation temperature and time, hexane volume and shaking time) were optimized in the esterification reaction with MeOH using H_2SO_4 as catalyst applying a basic simplex method. The initial values of these variables, as well as the minimum and maximum values between which they can move, were informed to the software that provides the experimental conditions and allows the sequential optimization.

A chromatographic procedure using gradient elution in ion-exchange chromatography was optimized using a basic simplex [43]. Specific software was applied to model the behavior of the chromatographic peaks. Parameters describing gradient profiles for the separation of the substances were based on the criterion obtained from calculated chromatograms. The simplex method was used to calculate

new gradient profiles in order to reach optimum separations for a selected set of analytes. Simplex optimization works stepwise and for each new combination of parameters that describe the gradient profile a new calculation is performed and from the calculated chromatogram the optimization criterion was determined. With this approach, it was feasible to reduce the time and cost of analyses of complex samples using this chromatography technique.

Giné et al. [49] have used a basic simplex for the optimization of an automated flow injection analysis procedure for chloride determination in water samples. Two experimental variables (flow rate and sample-to-reagent ration) were optimized and a fully automated manifold was used to control these variables. An interface allowed commanding pumping speed, proportions of reagent, sample introduction, on-time data acquisition and evaluation of responses by the simplex algorithm. The optimized system showed a sample throughput of 120 samples by hour.

A basic simplex was used in a reversed flow injection (rFI) system based on the chromogen reaction of the rhodanine assay for gallic acid by Phakthong et al. [40]. In order to improve the reproducibility and the sensitivity of the proposed rFI system, a variable-size simplex method was used to optimize three variables simultaneously (mixing coil length, concentration of rhodanine, and NaOH). The optimization process was finished after 18 experiments and, when compared with a traditional process using the one-variable-at-a-time method (OVATM), it became evident that the simplex method was much faster than the univariate one. After this optimization, the rFI system coupled with spectrophotometric detector was employed to quantify gallic acid in longan fruit extract samples.

Unlike works on the basic simplex, most publications in analytical chemistry are about studies employing the modified simplex, probably, because it offers characteristics and flexibility in the search for the optimal conditions in relation to the basic method, despite its higher operational complexity. With the use of specialized software in simplex optimization, the latter characteristic does not become a problem, requiring from the analyst only the correct data supply.

A variable size simplex methodology was used for the development of an analytical procedure by Ramalhosa et al. [38] to separate and quantify methylmercury and inorganic mercury by high-performance liquid chromatography coupled to cold vapor atomic fluorescence spectrometry (HPLC–CV–AFS). Six variables involved in this analytical system (flow rates of the mobile phase, water, reducing agent, carrier and shield argon, and the length of the reduction coil) were optimized using this modified algorithm. The evaluated response was the methylmercury fluorescence signal obtained for standard aqueous solutions' analysis. A total of 37 experiments were necessary to reach the optimum conditions and, using these values, the MeHg^+ signal was increased by a factor of 2.

Mendiguchia, Moreno and García-Vargas [37] have used a modified simplex to develop a method for copper determination from seawater by flame atomic absorption spectrometry after a preconcentration step based on the use of a bulk liquid membrane consisting of an organic solution containing di-(2-ethylhexyl) phosphoric acid (DEHPA) in kerosene. The studied variables were the pH of the source solution and the concentration of the buffer solution used to control it, the concentration of nitric acid in the receiving solution and the concentration of DEHPA in the organic phase. The permeability coefficient was used as response in the optimization process. Only 13 vertexes were necessary to reach the optimum conditions. Under these values, the preconcentration factor was 4.30.

Horstkotte et al. [42] applied the modified simplex methodology to optimize simultaneously four volumetric variables and two concentrations of one in-line prepared reagent of an SIA system in real time. The simplex optimization allowed approaching an ideal starting parameter set with a minimum of experiments. The developed analyzer was then used for the determination of dissolved oxygen in seawater samples from the Southern Ocean. The analytical system featured high signal

stability, robustness and a repeatability of 3% RSD (1st mode) and 0.8% (2nd mode) during shipboard application.

The modified simplex method was applied for the first time to determine compounds by a luminescence technique by Pulgarín, Molina and Pardo [36]. The method was based on the optimization of chemical (concentration of sulfuric acid and sodium sulfite) and instrumental (delay time, gate time and time between flashes) variables affecting phosphorescence using a geometric simplex in two and three space dimensions, respectively. The proposed procedure was satisfactorily applied to the determination of naftopidil in biological fluids (urine and serum). The application of the modified simplex provided a faster and simpler procedure to obtain optimal values of chemical and instrumental variables affecting the phosphorescence signal.

A modified simplex was employed to optimize mobile phase composition and post-column reaction by Horstkotte et al. [46] to evaluate the applicability of sequential injection chromatography for the determination of transition metals in water. The mobile phase consisted of 2,6-pyridinedicarboxylic acid as analyte-selective compound, sodium sulfate, and formic acid/sodium formate buffer. Post-column addition of 4-(2-pyridylazo) resorcinol was carried out for spectrophotometric detection of the analyte complexes at 530 nm. Optimization of the mobile phase composition was done by the modified simplex method, which included the following parameters: concentrations of PDCA, formic acid, and Na_2SO_4 and the pH value. The simplex optimization of peak resolution as desirability function was carried out. The method was successfully applied to the analysis of copper(II), zinc(II), and iron(II) cations in real samples of drinking (tap and bottled) and mineral waters.

Table 6 illustrates some recent applications of the simplex method in the optimization of analytical procedures and emphasizes some specific information such as the determined analytes' concentration, the types of samples, the analytical system utilized and the optimized parameters.

6. Trends for simplex optimization in analytical chemistry

Application of simplex optimization in analytical chemistry is still mainly limited to classical basic and modified algorithms. However, it is possible to note that some few published papers are pointing trends, such as multi-objective simplex optimization and hybridization of a classical simplex with other optimization methods.

Due to the fact that the simplex method is robust, easily programmable and fast, several researchers have developed some hybrid optimization schemes to be applied to several areas of science. Many attempts have been made to hybridize evolutionary algorithms (and their branches such as genetic algorithms) with the classical simplex methods [20]. Cai et al. [44] have used an optimization method based on the modified simplex method with the fuzzy set theory in the optimization of chromatographic separation and derivation conditions of amino acids neurotransmitters. Konstantinidis et al. [21] have applied a novel hybrid experimental simplex algorithm to identify "sweet spots" in bioprocess development using ion exchange chromatography.

Other demand still little explored in the application of the simplex methodology in the development of analytical methods is the multi-objective optimization. For chromatographic methods, for example, a problem for simplex optimization is the high amount of responses (retention times, resolutions between peaks, etc.) that need simultaneous treatment. To obtain a unique response, that could represent the quality of all peaks of the chromatogram, several chromatographic response functions (CRFs) have been proposed.

To optimize any analytical method it is necessary taking into account several of their performance characteristics, which might almost always be in conflict. Thus, some authors have proposed mathematical functions to attain multi-objective conditions and satisfy simultaneous responses [13]. A promissory alternative for multi-objective optimization is the use of Derringers' desirability functions [22], a very usual tool frequently applied in response surface methodology.

Table 6

Some recent applications of simplex optimization in analytical chemistry.

Analyte	Samples	Analytical system	Optimized variables	Reference
Co, Ni and Cr	–	Ion-pair reversed phase high performance liquid chromatographic separation of chelates of these three metals	Acetonitrile, buffer and surfactant concentrations	[18]
Mn	Vegetal leaves and natural waters	Automated preconcentration system coupled with FAAS	pH, flow rate and HCl concentration	[23]
Fe	Milk and waters	Flow injection on-line separation and preconcentration system	Variables associated with analyte retention and variables associated with analyte elution	[24]
Imidazolium and pyridinium ionic liquid cations	–	Polar reversed-phase—high performance liquid chromatography	Mobile phase composition	[25]
Formaldehyde	Urban rainwater	Modified Trautz–Schorigin reaction into a flow-injection analysis for chemiluminescence determination	Flow parameters	[26]
Mo	Coal fly ash, sediment, soil and urban dust	Slurry extraction-electrothermal atomic absorption spectroscopy	HNO ₃ and HCl concentrations	[27]
Amoxicillin and clavulanic acid	Pharmaceuticals	Sequential injection analysis with a diode-array detector and multivariate curve resolution with alternating least squares	HCl and NaOH concentrations, NaOH and sample volumes and flow rate	[28]
Amphetamine and amphetamine analogues	Seized illicit tablets	Capillary zone electrophoresis	pH and buffer concentration	[29]
Vitamins A and E	Pharmaceutical preparations	Micellar liquid chromatography	Percent of organic modifier, pH of the mobile phase, concentration of surfactant, and flow rate of the mobile phase	[30]
Nabumetone	Commercial pharmaceutical preparations	Micellar-stabilized room temperature phosphorescence using the stopped-flow mixing technique	Surfactant, Na ₂ SO ₃ , H ₂ SO ₄ , NaOH and TiNO ₃ concentrations	[31]
Polycyclic aromatic hydrocarbons (PAHs)	–	High performance liquid chromatography	Starting and ending compositions of acetonitrile and water, linear gradient time, mobile phase flow rate, column temperature and holding time of the final mobile phase composition	[32]
Tartaric acid	Wines	Flow injection manifold using a dialysis unit to eliminate matrix interferences and to accomplish on-line dilution and spectrophotometric determination	Residence time and sampling rate	[33]
Polycyclic aromatic hydrocarbons (PAHs), phthalate esters and polychlorinated biphenyls (PCBs)	Waters	Solid-phase microextraction coupled to gas chromatography mass spectrometry	Extraction time, extraction temperature, desorption time, desorption temperature and the weight of sodium chloride	[34]
Periodate and iodate	Waters	Flow injection analysis (FIA) coupled with spectrophotometric determination	Chemical and FIA variables	[35]
Antihypertensive drug (naftopidil)	Urine and serum	Heavy atom induced room temperature phosphorescence	Chemical and instrumental variables affecting phosphorescence	[36]
Cu	Seawater	Bulk liquid membrane system to metal preconcentration and determination using flame atomic absorption spectrometry	pH and chemicals concentration	[37]
Methylmercury (MeHg) and inorganic mercury	Sediment certified reference material	High-performance liquid chromatography coupled to cold vapor atomic fluorescence spectrometry	Mobile phase, Water, Reducing agent, Argon carrier and Argon shield flow rates and Reduction coil length	[38]
Phenoxy acid herbicides and 2,4-D esters	Soil	Gas chromatography with flame ionization detector	Methylation temperature, methylation time, hexane volume and shaking time	[39]
Gallic acid	Longan samples	Reverse flow injection analysis with colorimetric detection	Conditions involved in colorimetric reaction and FIA parameters	[40]
Glucose	Glucose solutions	Glucose electrodes based on glucose oxidase immobilized in an osmium redox polymer	Enzyme concentration and the rate constant <i>k</i>	[41]
Dissolved oxygen	Seawater	Analyzer system based on sequential injection analysis (SIA)	Volumetric and chemicals variables	[42]
Eighteen organic and inorganic ions	Standard solutions	Ion exchange chromatography	Parameters describing profile for the isocratic separation in ion chromatography	[43]
Risperidone, 9-hydroxyrisperidone, monoamine and amino acid neurotransmitters	Human urine	Ultra-performance liquid chromatography–electrospray ionization tandem mass spectrometry	Variables involved chromatographic separation and dansyl derivatization conditions	[44]
Medium and long-chain free fatty acids	Cooked cured pork ham exudates	Stir-bar sorptive extraction (SBSE)	Variables involved to conditions of extraction	[45]
Transition metal cations (Cu ²⁺ , Zn ²⁺ and Fe ²⁺)	Natural water samples	Sequential Injection Ion Chromatography	Mobile phase composition and post-column reaction	[46]
Basic red 46 textile dye	Synthetic solutions prepared with non-purified commercial dye	Spectrophotometric measures at 532 nm of residual dye in the solution after adsorption	Variables involved in the conditions of the active carbon preparation	[47]
Cd	Sediments, food and cigarettes	Flow injection solid-phase extraction coupled to flame atomic absorption spectrometry	Optimization of flow parameters (pH and sampling flow rate)	[48]

7. Conclusions

The simplex optimization consists of a practical and reliable method for the development of analytical methods without the need for more complex mathematical-statistical treatments. Simplexes – especially those with basic and modified algorithms – have been widely applied to find the best experimental conditions to perform analytical procedures and hence ensure good analytical characteristics such as higher sensitivity and good accuracy, among others.

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