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Optimization by means of responses surface of an analytical sequence using a sequential injection system

Alberto Pasamontes *, Mª Pilar Callao

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Marcel lí Domingo s/n, Campus Sescelades, 43007 Tarragona, Spain

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Abstract

An experimental design method was applied to determine the optimum working conditions for sequential injection analysis (SIA) to obtain second-order data that will be treated using multivariate curve resolution with alternating least squares (MCR-ALS).

The critical step is to design an analytical sequence that provides relevant information. This sequence depends on parameters related to the system, the chemical reaction, and the chemometric treatment of the data. Also, from the multiple responses that quantify the quality of this analytical sequence, a single response is determined from the desirability function.

This method involves a factor-screening step, in which both the global desirability function and the individual responses are considered and a response surface-modelling step, in which the most relevant factors are considered.

Keywords: Experimental design; Desirability function; MCR-ALS; SIA; Amoxicillin; Responses surface

1. Introduction

Currently available analytical systems generate various sizes of data: zero-order data, when the signal obtained for each sample is a single datum; first-order data, when a vector is generated; and second-order data, when a matrix is generated. These second-order data are very interesting because, using suitable chemometric treatment and from one analysis, we can obtain qualitative information about the sample or/and quantitative information about the analytes in the presence of interferents [1].

Several instrumental configurations provide second-order data, e.g. chromatographic or flow techniques with amperometric detectors, UV–vis with a diode-array spectrophotometer detector (DAD) and fluorimetric spectrophotometric detector [2–5].

If we use sequential injection analysis (SIA) [6] with a DAD, the sample and reagent zones are sequentially aspirated into a channel using a selection valve to subsequently reverse the flow and transport the stacked zones into the detector. During the course of these operations, the zones undergo some mutual dispersion and the analyte interacts with the reagents, evolving into another species to obtain a matrix data.

In previous papers [7,8], we observed that the critical step to determine amoxicillin in pharmaceuticals is to design an analytical sequence that not only provides second-order data (to generate an evolving system) but also, in the final results of the process, we have to visualize all the analytes and the quantification error must be optimum. Finding this analytical sequence depends on factors related to the system, the chemical reaction, and the chemometric treatment of the data.

In this study, we will apply experiment design methods to find the optimum response for determining amoxicillin in pharmaceuticals. First, we screened the factors and with the selected factors, we made a central composite design to obtain a response surface. To define the quality of the analytical sequence we need to have several responses. To reduce the number of responses to only one, we use the desirability function. We use like a chemometric tool multivariate curve resolution using alternating least squares (MCR-ALS) [9]. Unlike other chemometric treatments, such as classical least squares (CLS), MCR-ALS does not need to know the composition of the interferents. This

* Corresponding author. Tel.: +34 977558122; fax: +34 977558446.
E-mail address: alberto.pasamontes@urv.net (A. Pasamontes).

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characteristic is known as the advantage of second-order data [10].

2. Experimental method

2.1. Reactive

We prepared amoxicillin and sodium hydroxide stock standard solutions by weighing the required amount of the respective compounds (amoxicillin from Sigma and sodium hydroxide from Prolabo) and dissolved them in purified water (from a Milli-Q water system from Millipore). The pharmaceuticals drug was augmentine (500 mg of amoxicillin per packet) from SmithKline Beecham, S.A.

2.2. Apparatus

The sequential injection analyser comprised: CAVRO XL 3000 stepper motor-driven syringe pump connected to the PC with an RS-232 interface; a 6-position Eurosas EPS 1306 BPB automatic valve connected to the computer through a PCL-711S PC-Lab-Card; omnifit PTFE tubing reaction coil: 70 cm × 0.8 mm; holding coil: 200 cm × 0.8 mm; an HP8452A diode-array spectrophotometer controlled by an HP Vectra 5/75 computer equipped with an HP-IB IEEE 488 interface for communications; a Hellma 178.711QS flow-through cell. The spectra were recorded every 2 nm in the 220–340 nm range, with an integration time of 0.1 s. The spectra measurements were taken every 0.7 s.

2.3. Software

HP89531A software was used to record and store the spectra. Customized software was used to control the SIA. All calculations relating to MCR-ALS were performed with laboratory-written software under a MATLAB 5.3 computer environment [11]. This software is available from the authors [12]. The adjustment and optimisation of the response surfaces for the desirability function were done with NemrodW [13].

2.4. Chemical reaction [7,8]

The acid–base properties of amoxicillin (pK_a: 2.4, 7.4, 9.01, 10.29) and its spectral characteristics enable us to generate an evolving system that leads to a pH gradient between the pH of the aqueous solution of amoxicillin (pH 4.5) and basic pH. The spectra of amoxicillin in its acid ($amox_{ac}$) and basic ($amox_{bas}$) forms are shown in Fig. 1b. The spectrum indicated by a dashed line (called the acid species of amoxicillin ($amox_{ac}$)) was obtained by measuring the amoxicillin in hydrochloric acid (pH 1), in water (pH 4.5) and in a buffer of NaH_2PO_4/Na_2HPO_4 (pH 7.5). The spectrum indicated by a continuous line (called the basic species of amoxicillin ($amox_{bas}$)) was obtained by measuring the amoxicillin in a buffer of NH_3/NH_4Cl (pH 10.1) and in sodium hydroxide (pH 13). The proposed chemical system is:

$$\underbrace{Amox_{ac} + NaOH}_{1} \xrightarrow{\Delta t} \underbrace{Amox_{ac} \rightleftarrows Amox_{bas}}_{2}$$

This system includes a dynamic part (step 1), which corresponds to the interdiffusion between NaOH and $amox_{ac}$, which

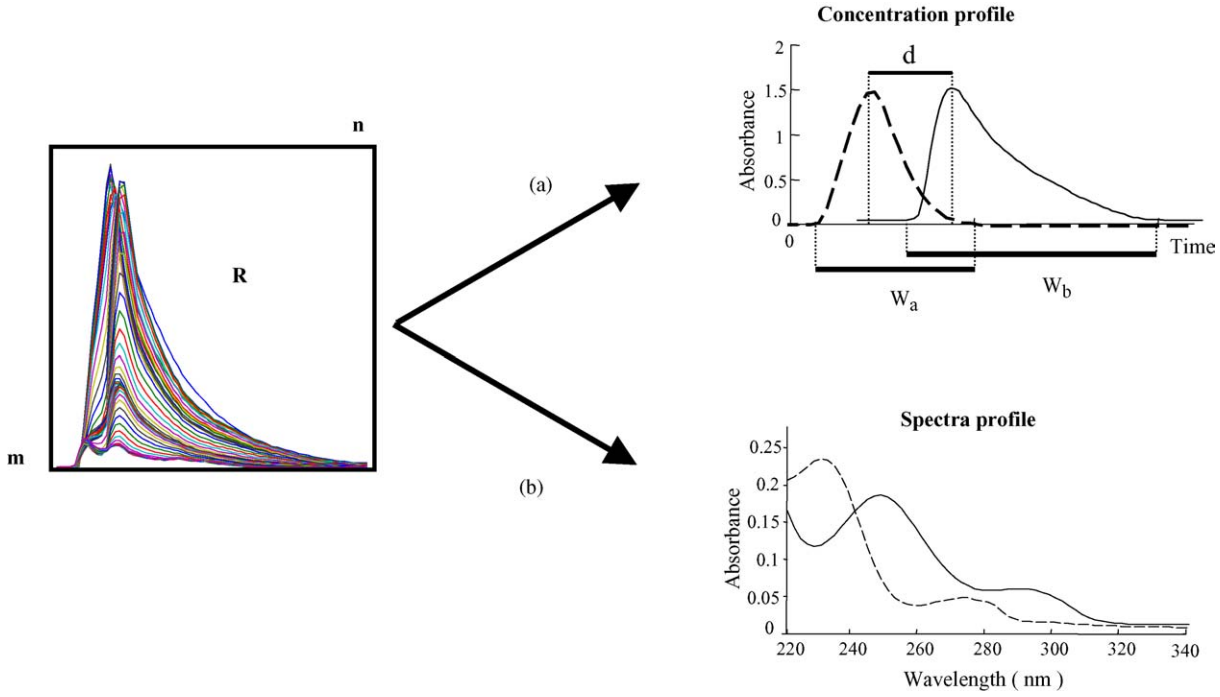

Fig. 1. Result obtained by MCR-ALS. *R* corresponds to raw matrix, where *m* is the number of spectra recorded over time and *n* is the number of wavelengths. (a) Matrix of concentration profiles and (b) spectra profiles.

involves a pH gradient into the reactor coil of the SIA, and an equilibrium part (step 2) between $amox_{bas}$ and $amox_{ac}$, which is established according to the pH in the various zones of the reactor coil.

3. Data treatments

3.1. Application of MCR-ALS

The aim goal of MCR-ALS is to decompose the raw matrix, whose columns represent the wavelengths and whose rows represent the times at which the measurements were taken, into the product of two matrices; one matrix will give information about concentration profiles and the other matrix will give information about the spectra profiles of every component. The final results of applying MCR-ALS are shown in Fig. 1. The chemometric tool used for this process is: principal component analysis (PCA) [14] to fix the number of species and simple-to-use interactive self-modelling mixture analysis (SIMPLISMA) [15] to make the initial estimation. We can also start ALS using the pure spectrum, but an initial estimation of the spectra of the interferents is needed.

Depending on the nature and structure of the data, different constraints can be applied during the ALS optimization. Another way to improve the resolution is to use augmented matrices by columns, which involves adding one or more matrices that have one or two orders in common with new information [16].

3.2. Evaluation of the responses

To find an optimal analytical sequence, we must select a response, or responses, that reflect the quality of the results. In our study, we aimed to achieve a good resolution between species, but this qualitative response had to be transformed into quantifiable responses. The quantifiable responses we considered were:

(1) The resolution of the concentration profile (R_s), which, because of its similarity to the chromatographic process, we evaluated using the following expression:

$$R_s = \frac{2d}{W_a + W_b} \tag{1}$$

where d is the distance between the maxima of the peaks of the concentration profiles (Fig. 1a) of the acidic and basic species, and W_a and W_b are the widths of the two peaks.

(2) The correlation between the spectra obtained in the resolution process and the spectra of the pure species.

(3) The lack of fit (lof) of the model from experimental data was evaluated from the following equation:

$$\text{lof} = \sqrt{\frac{\sum_{i,j}(d_{ij} - \hat{d}_{ij})^2}{\sum_{i,j} d_{ij}^2}} \tag{2}$$

where d_{ij} are the corresponding values of the raw data matrix and $\hat{d}_{ij}$ are the corresponding values calculated after the optimization process (ALS).

(4) The quantification error was evaluated from the following equation:

$$\text{Error} = \frac{|r_{exp} - r_{theo}|}{r_{theo}} \times 100 \tag{3}$$

From the areas obtained in the resolution process with augmented matrices (samples + reference standard), we get the relative area r_{exp}

$$r_{exp} = \frac{a_s}{a_{rst}} \tag{4}$$

where a_s is the area of the sample of amoxicillin and a_{rst} is the area of the reference standard. Ideally, r_{exp} should be equal to the relation between the concentration of the analyte in the sample (c_s) and the concentration of the analyte in the reference standard (c_{rst}). We thus define r_{theo}:

$$r_{theo} = \frac{c_s}{c_{rst}} \tag{5}$$

3.2.1. Desirability function [17,18]

When there are multiple responses to evaluate, an overall desirability function is suitable. The overall desirability function, D, is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions. The weight of the individual functions reflects the importance of each response. The expression that defines the overall desirability function is:

$$D = \sqrt[n]{d_1^{p1} d_2^{p2} \cdots d_n^{pn}} \tag{6}$$

where p_i is the weight of the response, n the number of responses and d_i is the individual desirability function of each response obtained from the transformation of the individual response of each experiment. At this stage, the value $d_i = 1$ is assigned when all the previous specifications are fully met and the value $d_i = 0$ is assigned when they are not. Values between 0 and 1 are obtained using a continuous function of the measured response.

3.3. Evaluation of the factors

The factors associated with the system and the chemical reaction were: (a) the flow, which directly influences the time spent in the channel and the interdiffusion of sodium hydroxide and amoxicillin; (b) the volume of sodium hydroxide and amoxicillin, which must be enough to produce the reaction and generate the pH gradient correctly; (c) the concentration of sodium hydroxide, since it affects the pH interval that can be obtained in the reactor. Also, the interval achieved must ensure the presence of the two species of amoxicillin; (d) the concentrations of amoxicillin in the sample.

The factors associated with MCR-ALS were: (i) the concentration of the reference standard, and (ii) whether to impose conditions of trilinearity when treating the data [8].

The choice of the domain of the quantitative factors can be evaluated from previous experiments. The experimental domain of these factors is shown in Table 1.

Table 1
Factors and experimental domain

Factors	Domain	
	Low	High
V. amox[a]	0.13	0.22
V. NaOH[b]	8	42
[Amox][c]	50	300
[NaOH][d]	0.01	0.5
Flow[e]	0.5	2.5
Type of data	Trilinearity	No trilinearity
Ref. Stand.[f]	50	300

[a] Volume of amoxicillin (ml).
[b] Volume of sodium hydroxide (ml).
[c] Concentration of amoxicillin (μg/l).
[d] Concentration of sodium hydroxide (mol/l).
[e] Flow (ml/min).
[f] Concentration of amoxicillin in reference standard (μg/l).

3.4. Experiments design: [19]

3.4.1. Screening design

With a limited number of experiments, screening designs evaluate how a large number of factors affect the response. The most common screening designs are two-level fractional saturated designs and Plackett–Burman designs. The effects of the factors can be evaluated using a Pareto chart, which shows important factors in the response in the form of a graph.

3.4.2. Central composite design

The second-order polynomial model is usually suitable for estimating the experimental response and finding the optimal point. One of the designs that can be used to optimise the second-order response surface is the central composite design. This type of experiment design includes a full factorial 2^k (where k is the number of factors), a series of replications in the centre and points centred on the faces with a pre-determined axial distance.

4. Results and discussion

Table 2 shows the responses of our experiments when we applied Plackett–Burman design. The bold values indicate the maximum and minimum values got of each response that fixes the range of the factors in the responses. For responses such as lack of fit or quantification error, the maximum value of response is assigned the value of $d_i = 0$ and the minimum value of response is assigned the value of $d_i = 1$. For responses such as the resolution of the concentration profile, the correlation between the spectra obtained in the resolution process and the spectra of the pure species for acidic and basic species, the maximum value of response is assigned the value of $d_i = 1$ and the minimum value of response is assigned the value of $d_i = 0$. The individual desirability values for experimental values between these limits are linear between 0 and 1. Overall desirability was obtained from Eq. (6). We considered that all the responses were equally important so, to obtain overall desirability, they were not weighted.

From the overall and individual desirability functions, we obtained the Pareto chart, which shows the most important factors. Fig. 2 shows the Pareto chart with the overall desirability function. We can see that the only important factor was the volume of the sample with a p-value of 10%. The next two factors (volume of NaOH and flow) had p-values of 30%. However, when we studied the experimental responses individually, we found that the p-values of these two factors were around 10%. For the volume of NaOH the responses were the lack of fit and the correlation between the basic spectra. For the flow, the responses were the lack of fit and the correlation between the acid spectra and the resolution.

Table 2
Responses of Plackett–Burman design

Experiment no.	lof[a]	Quan. E[b]	R_s[c]	Cor. acidic[d]	Cor. basic[e]
1	**2.4**	0.6	0.13	**0.998**	**0.991**
2	3.7	**0.3**	0.15	0.967	0.988
3	5.6	31.3	0.13	0.700	0.979
4	2.9	31.6	0.07	0.996	0.849
5	8.2	3.2	0.20	0.975	0.991
6	4.6	36.8	**0.04**	0.942	**0.441**
7	**16.2**	15.0	0.28	0.983	0.946
8	13.7	24.0	0.26	0.984	0.931
9	9.2	1.0	**0.29**	0.919	0.989
10	3.7	0.4	0.26	0.991	0.975
11	7.5	**40.0**	0.08	**0.656**	0.966
12	4.4	1.9	0.23	0.991	0.831

[a] Lack of fit of model.
[b] Quantification error.
[c] Resolution of the peak.
[d] Correlation between the spectra of acidic species obtained in the resolution process and the spectra of the pure acidic species.
[e] Correlation between the spectra of basic species obtained in the resolution process and the spectra of the pure basic species.

As an example, Fig. 3 shows the spectra and concentration profile we obtained after applying MCR-ALS. Fig. 3a shows the result of a good resolution and Fig. 3b shows the result of a bad resolution. At the top of each figure, we can see the concentration profile (augmented matrix made up of the augmentine sample and a standard of 60 mg/l). At the bottom of each figure, we can see the pure spectra.

To evaluate the response surface we considered the first three factors in the Pareto chart. For the other factors, we fixed those that provided the best values for the overall desirability function

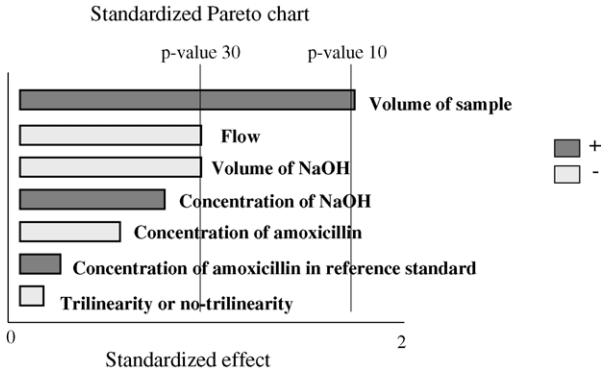

Fig. 2. Pareto chart of the desirability function from the responses in the Plackett–Burman experiments.

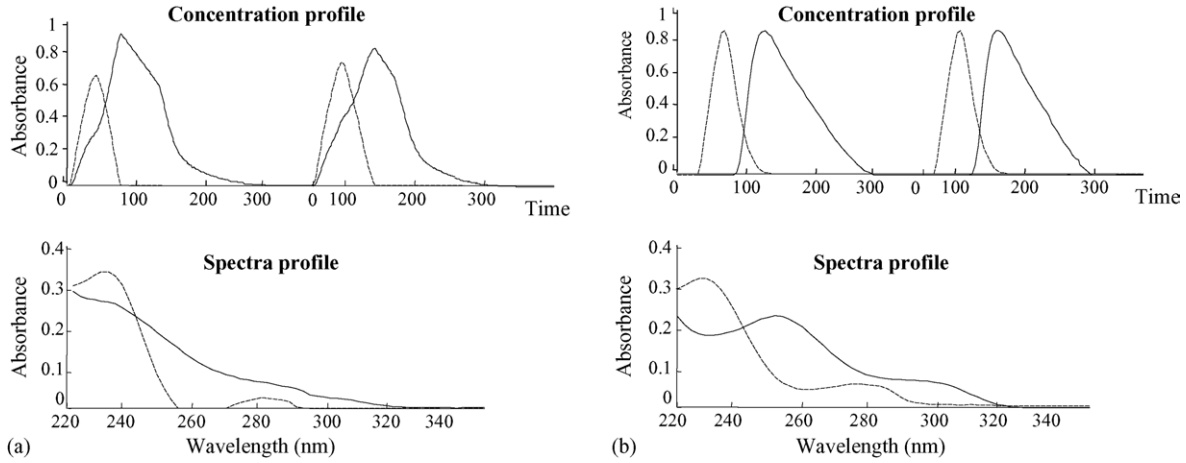

Fig. 3. Results obtained after applying MCR-ALS: (a) a good resolution and (b) a bad resolution.

(the concentration of sodium hydroxide was 0.5 M; the concentration of amoxicillin was 50 μg/l; and the concentration of reference standard was 300 μg/l) and imposed the no trilinearity condition on the data.

With the chosen factors we carried out an experiment corresponding to a central composite design 2^3, with four replications in the central point and with the points concentrated on the faces with an axial distance of one. See Table 3, where the first column is the number of experiments, the next three columns correspond to the previously selected factors (flow, volume of sodium hydroxide and volume of amoxicillin, respectively) and the last five columns correspond to the responses. Experiments 15–18 were performed in the central point, which enabled us to estimate the experimental error.

After obtaining the results, we transformed the responses into individual desirability. The conditions for obtaining these values are given in Table 4. The first column indicates whether we wished to maximise or to minimise each of the responses. The next two columns show the experimental maximum or minimum values of the responses. The next two columns show the upper and lower limits we chose to apply the desirability function. These limits are more restrictive than the experimental ones because the aim is not to show the influence of the factors but to establish a response surface in a range where the global desirability function will be optimum.

Table 4
Limits of experimental values for applying desirability function

	Goal	Response range[a]		Transformation range[b]	
		Low	High	Low	High
Cor. acidic	Max.	0.950	0.992	0.970	0.990
Cor. basic	Max.	0.270	0.980	0.970	0.990
lof	Min.	5.200	19.280	6.000	14.000
Quan. E	Min.	0.018	13.170	4.000	10.000
R_s	Max.	0.070	0.340	0.240	0.290

[a] Experimental responses range.
[b] Transformation of experimental responses range.

After setting these limits, we calculated the overall desirability function for each experiment. We adjusted these desirability functions to a response surface that provides an equation depend-

Table 3
Experiments and responses of the central composite design

Exp. no.	Flow	V. NaOH	V. amox	lof	Quan. E	Cor. basic	Cor. acidic	R_s
1	0.5	8	0.13	14.2	1.53	0.98	0.27	0.07
2	2.5	8	0.13	10.7	1.82	0.95	0.34	0.23
3	0.5	42	0.13	16.6	0.40	0.98	0.97	0.26
4	2.5	42	0.13	5.9	1.23	0.98	0.98	0.32
5	0.5	8	0.22	19.3	1.70	0.97	0.77	0.32
6	2.5	8	0.22	11.3	2.00	0.98	0.81	0.29
7	0.5	42	0.22	14.3	2.43	0.98	0.96	0.30
8	2.5	42	0.22	5.2	1.01	0.98	0.97	0.22
9	1.5	25	0.22	8.5	1.97	0.98	0.92	0.17
10	1.5	25	0.13	10.3	13.17	0.99	0.95	0.26
11	1.5	42	0.18	7.9	7.70	0.99	0.97	0.23
12	0.5	25	0.18	18.3	1.87	0.98	0.94	0.34
13	1.5	8	0.18	18.1	8.98	0.99	0.75	0.32
14	2.5	25	0.18	7.7	1.41	0.98	0.95	0.25
15	1.5	25	0.18	9.1	0.60	0.99	0.94	0.22
16	1.5	25	0.18	8.9	0.61	0.98	0.93	0.25
17	1.5	25	0.18	9.1	0.61	0.98	0.93	0.22
18	1.5	25	0.18	9.2	0.62	0.98	0.93	0.21

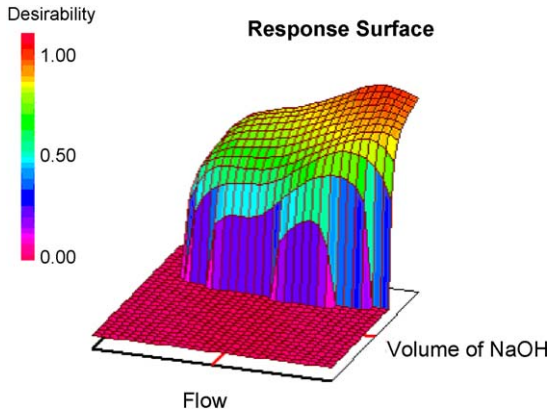

Fig. 4. Response surface (desirability) for volume of NaOH and flow. The third factor, the volume of amoxicillin is 0.175 ml.

ing on three factors and where the response will be the overall desirability function. The mathematical equation that represents this response surface is:

$$y = 0.458 + 0.072x_1 + 0.556x_2 + 0.295x_3 - 0.014b_{12}x_1x_2 - 0.021x_1x_3 - 0.421x_2x_3 - 0.314x_1^2 - 0.036x_2^2 - 0.05x_3^2$$

where y is the global desirability function, x_1 the flow, x_2 the volume of NaOH and x_3 is the volume of the sample. To graphically represent this equation we have to fix a factor, e.g. the volume of amoxicillin, and represent a response surface (see Fig. 4) for the flow and volume of sodium hydroxide factors.

As the maximum of this response surface represents the highest value of the overall desirability function, we have the optimum conditions. These high values of overall desirability function are achieved for high values of flow and volume of sodium hydroxide. If the value of the overall desirability function is zero, and the values of flow and volume of sodium hydroxide are low, at least one of the responses is outside the interval permitted.

5. Conclusions

For a system made up of SIA and MCR-ALS, an attractive way to find an optimal analytical sequence that can be used to carry out a quantitative or qualitative analysis of the sample is to make a response surface. To obtain this response surface we need to use a correct experimental design and a desirability function.

Two important steps are: (i) to choose the correct responses that reflect the quality of the results and transform these responses into quantifiable responses, and (ii) to define the experimental domain and correctly set the values of desirability.

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References

[1] K.S. Booksh, B.R. Kowalski, Anal. Chem. 66 (1994) 782A.
[2] M.C. Antunes, J.E.J. Simao, A.C. Duarte, R. Tauler, Analyst 127 (2002) 809.
[3] M. Sena, J.C. Fernandes, L. Rover, R.J. Poppi, L. Kubota, Anal. Chim. Acta 409 (2000) 159.
[4] J. Saurina, R. Tauler, Analyst 125 (2000) 2038.
[5] J. Saurina, S. Hernandez-Cassou, R. Tauler, Anal. Chem. 69 (1997) 2329.
[6] J. Ruzicka, G.D. Marshall, G.D. Christian, Anal. Chem. 62 (1990) 1861.
[7] A. Pasamontes, M.P. Callao, Anal. Chim. Acta 485 (2003) 195.
[8] A. Pasamontes, M.P. Callao, Anal. Chim. Acta 515 (2004) 159.
[9] R. Tauler, A.K. Smilde, B.R. Kowalski, J. Chem. 9 (1995) 31.
[10] E. Sánchez, B.R. Kowalski, J. Chemomet. 2 (1990) 247.
[11] Matlab, The Mathworks, South Natick, MA, USA.
[12] R. Tauler, A. de Juan, Multivariate curve resolution home page, http://www.ub.es/gesq/mcr/mcr.htm.
[13] D. Mathieu, J. Nony, R. Phan-Tan-Luu, NemrodW, Version 2000, LPRAI, Marseille, 2000, http://www.nemrodw.com.
[14] E.R. Malinowski, Factor Analysis in Chemistry, Wiley, New York, 1991.
[15] J.M. Phalp, A.W. Payne, W. Windig, Anal. Chim. Acta 318 (1995) 43.
[16] J. Mendieta, M.S. Diaz-Cruz, R. Tauler, M. Esteban, Anal. Biochem. 240 (1996) 134.
[17] G. Derringer, R. Suich, J. Qual. Technol. 12 (1980) 214.
[18] E.C. Harrington, Ind. Qual. Control (1965) 494.
[19] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Elsevier, Amsterdam, 1998.