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Determination of the capability of detection of a hyphenated method: application to spectroelectrochemistry

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Abstract

A procedure to evaluate the capability of detection of a second-order analytical technique to determine an analyte in presence of an interferent has been proposed taking into account α and β errors in a similar way as ISO norms indicate for the univariate analytical methods. The potentiality of spectroelectrochemistry as a quantitative three-way technique of analysis has been analysed. Trilinearity of spectroelectrochemical data has been studied since it is a necessary condition to apply the trilinear decomposition (TLD) method. As an example, the voltabsorptometric determination of *o*-tolidine in presence of high concentration of ferrocyanide was chosen to test the applicability of the proposed method. In the same way, the capability of discrimination has been determined. In addition, a second-order standard addition method (SOSAM) has been applied to calculate the concentration of the analyte of interest in the presence of this interferent, avoiding the need to previously identify and determine the quantity of the interferent.

Keywords: Three-way analysis; TLD; Capability of detection; SOSAM; Spectroelectrochemistry; OTTLE; *o*-Tolidine; Ferrocyanide

1. Introduction

The capability of detection of a method of analysis is one of the figures of merit that evaluate its performance [1]. This figure of merit is well defined for univariate calibration in the ISO 11843-1 [2] and more recently in the ISO 11843-2 [3], but there is not yet a general accepted definition for first-order data and much less for second-order data, although several frameworks have been proposed in the bibliography [4,5,6]. First-order instruments generate multiple measurements for one sample (e.g. spectrometers or chromatographs), whereas second-order instruments are those that generate a matrix of data per sample [7]. Present-day demand in the determination of more and more complex samples and the great development of analytical technology make the use of second-order instruments, related to the so-called hyphenated techniques, more and more common and essential, since they greatly increase the efficiency of the chemical analysis. Among them, some of the most usual techniques are GC/MS, GC/FTIR, HPLC/UV, kinetic spectrophotometric analysis, etc., and also others, even though they are less widespread, such as spectroelectrochemistry.

Spectroelectrochemistry has been widely applied to inorganic, organic and biological systems [8,9]. Spec-

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troelectrochemical experiments have been used in order to elucidate reaction mechanisms, and to determine rate constants, diffusion coefficients, standard potentials and number of electrons involved in electrochemical reactions. In fact, most of the revised bibliography in the field of quantitative spectroelectrochemistry under restricted diffusion in optically transparent thin-layer electrodes (OTTLEs) is based mainly on establishing the analogies between the voltabsorptometric response and the well known current/potential signals through the use of derivative techniques [10,11,12]. The aim of these works is to find out the equivalence between the dA/dt vs. E curves and the voltammetric wave in such a way that the former can be analysed in a conventional way to, through them, determine some of the quantitative parameters previously indicated. This is due to the fact that, as a rule, the current curves obtained from the voltabsorptometric signal are of much better quality than the original since the capacitive background currents do not contribute to the absorbance.

The good quality of these signals makes them really interesting to be analysed with quantitative purposes, but there have been few analytical applications in which the concentration of the analytes was determined. In addition, in these cases, only a small portion of the information provided by the hyphenated technique is used except for some very specific papers [13], although today, several chemometric methods have been developed that allow one to use all this large quantity of data.

In this sense, several three-way methods have been developed [14] with a common characteristic called 'second-order advantage', which implies that the analysis can be performed in the presence of unknown interferents [15,16]. Three-way analysis has already been applied to analyse complex samples where unknown interferents are present, especially in the field of hyphenated spectroscopic and chromatographic techniques [17,18,19,20]. Among these methods, trilinear decomposition method (TLD) [21] is usually considered the method of choice for strictly trilinear three-way data of low rank, providing a unique solution, which coincides with the real one when the system is trilinear. This method has been used to analyse several kinds of data, such as data from a second-order fiber optic heavy metal sensor [22], stopped-flow kinetic data for binary amino acid mixtures [23], data provided by a kinetic florescence detection method for TLC separations [24], etc. However, there are no applications of TLD to voltabsorptometric data, despite the fact that trilinearity of this kind of data has already been pointed out [25]. In this paper, the suitability of spectroelectrochemical data for being analysed with TLD is shown.

Using this method to decompose the three-way array of data, a procedure is proposed in this paper to evaluate the capability of detection of a second-order technique of analysis, which implies the consideration of errors of kind I (false positive, α) and kind II (false negative, β) in a similar way as the ISO norm indicates for univariate methods [2,3]. In addition, the paper shows that the capability of discrimination [27] of a second-order technique can also be determined by using this decomposition method.

In this paper, we will use the notation recently introduced by Harshman [26] that generalizes the capabilities of matrix notation and algebra to n-way arrays.

As an example, the procedure proposed has been applied to a spectroelectrochemical analysis under restricted diffusion in OTTLE. The capability of detection and the capability of discrimination have been estimated in the voltabsorptometric determination of *o*-tolidine in the presence of potassium ferrocyanide. The latter compound works as an interferent on both analytical domains of the *o*-tolidine voltabsorptometric signal because both the voltammetric signal and the spectrum of potassium ferrocyanide overlap the *o*-tolidine signals. Likewise, a second-order standard addition method [28], SOSAM, has been performed with the same analytical system without the need to simultaneously calibrate the interferent.

2. Theory

2.1. Trilinearity of voltabsorptometry

Voltabsorptometry consists of measuring the radiation absorbed in the UV–VIS region of the spectrum by the species of interest that are simultaneously oxidized or reduced in an electrochemical cell by varying the applied potential, E. This greatly enhances the information provided by the electrochemical experiment by the addition of spectral information

about material at the electrode surface, resulting in a two-dimensional matrix of data per sample. Next, a study of the relation of the voltabsorptometric signal is made in order to analyse the relation expected between the experimental response, absorbance, and the concentration of the analytes taking into account the electrochemical process.

The mass balance describing a voltammetric reversible reaction

$$\mathrm{Ox} + ne^- \leftrightarrows \mathrm{Red} \tag{1}$$

is given by [29]

$$c = [\mathrm{Ox}] + [\mathrm{Red}] = c\frac{P(E)}{1+P(E)} + c\frac{1}{1+P(E)} \tag{2}$$

where c is the bulk concentration of the reactant and $P(E)$ is defined by

$$P(E) = \exp\left[\frac{nF}{RT}(E - E^{0'})\right]$$

So, the molar fractions for both Ox and Red species at the electrode as a function of potential are

$$x_{\mathrm{Red}}(E) = \frac{1}{1+P(E)} \tag{3}$$

$$x_{\mathrm{Ox}}(E) = \frac{P(E)}{1+P(E)} \tag{4}$$

The equation describing the i–E curves for an ideal thin-layer electrode, where concentrations are uniform, is given by

$$i(E) = n^2F^2\upsilon Vc\frac{P(E)}{RT(1+P(E))^2} \tag{5}$$

where V is the cell volume and υ is the scan rate ($\mathrm{d}E/\mathrm{d}t$).

If Eq. (5) is integrated with respect to time, it results

$$Q_{\mathrm{Red}}(E) = nFVcx_{\mathrm{Red}}(E) \tag{6}$$

$$Q_{\mathrm{Ox}}(E) = nFVcx_{\mathrm{Ox}}(E) \tag{7}$$

where $nFVc$ corresponds to the charge required to reduce or oxidize completely the reactant contained in the thin-layer cavity, and the other factors give the fraction of total charge consumed as a function of potential.

It is usual in voltammetry to identify the electroactive substances by their potential, $E_{\max}$, at which the extreme of current is achieved. When Eqs. (6) and (7) are compared with Eq. (5), it results that, at the same potential, the extreme of $(\mathrm{d}x/\mathrm{d}t)(E)$ is also reached. This is important to identify the mathematical factors of the trilinear decomposition with the chemical species.

If an OTTLE is used, it is possible to record a series of spectra at the same time as potential changes. The absorbance measured vs. both potential and wavelength is related to the charge produced in the redox reaction and is given by [30]

$$A_{\mathrm{Red}}(E,\lambda) = clx_{\mathrm{Red}}(E)\varepsilon_{\mathrm{Red}}(\lambda) \tag{8}$$

$$A_{\mathrm{Ox}}(E,\lambda) = clx_{\mathrm{Ox}}(E)\varepsilon_{\mathrm{Ox}}(\lambda) \tag{9}$$

where l is the thickness of the electrode, and $\varepsilon_{\mathrm{Red}}$ and $\varepsilon_{\mathrm{Ox}}$ are the molar absorptivities for the reduced and oxidized forms. As consequence of Eqs. (8) and (9), if I successive potentials ($E_1,\ldots,E_I$) are applied to a sample of concentration c, and absorbances are recorded for each potential at J wavelengths ($\lambda_1,\ldots,\lambda_J$), it results a matrix with I rows (one for each potential) and J columns (one for each wavelength). This matrix is the tensorial product of the I-dimensional vector that describes the voltammetric profile

$$\underline{x}_{\mathrm{Red}_I} = (x_{\mathrm{Red}}(E_1),\ldots,x_{\mathrm{Red}}(E_I)) \tag{10}$$

$$\underline{x}_{\mathrm{Ox}_I} = (x_{\mathrm{Ox}}(E_1),\ldots,x_{\mathrm{Ox}}(E_I)) \tag{11}$$

with the J-dimensional vector that defines the spectroscopic profile

$$\underline{y}_{\mathrm{Red}_J} = (\varepsilon_{\mathrm{Red}}(\lambda_1),\ldots,\varepsilon_{\mathrm{Red}}(\lambda_J)) \tag{12}$$

$$\underline{y}_{\mathrm{Ox}_J} = (\varepsilon_{\mathrm{Ox}}(\lambda_1),\ldots,\varepsilon_{\mathrm{Ox}}(\lambda_J)) \tag{13}$$

and as consequence of this

$$\underline{A}_{\mathrm{Red}_{IJ}} = cl\underline{x}_{\mathrm{Red}_I}\underline{y}_{\mathrm{Red}_J} \tag{14}$$

$$\underline{A}_{\mathrm{Ox}_{IJ}} = cl\underline{x}_{\mathrm{Ox}_I}\underline{y}_{\mathrm{Ox}_J} \tag{15}$$

The voltammetric profiles correspond to the charge related to the generation of the reduced and oxidized forms, respectively, when potential varies, and they will be mathematically identified. The derivatives of these vectors provide the computed voltammetric profiles, free of capacitive background contributions since they are obtained from the spectroscopic response instead of from the electrochemical (Eq. (5)). On account of the relationship between Eqs. (6) and (7) and Eq. (5), the identification of an electroactive substance should be done by means of the maximum/minimum of the derivative vector.

Eqs. (14) and (15) establish the trilinear character of voltabsorptometry. If one considers K samples of concentrations $z_1,z_2,\ldots,z_K$, or that is the same with concentration profile z_K, that is $z_K=(z_1,\ldots,z_K)$, and on each sample, the previous voltabsorptometric procedure is carried out, it results a three-way array of data composed by K matrices of dimension $I\times J$ with a generic element that, except for a constant factor, is

$$r_{IJK} = x_I y_J z_K \tag{16}$$

In practice, given a sample, they exist N species with voltammetric (x_{In}), spectroscopic (y_{Jn}) and concentration (z_{Kn}) profiles in such a way that the corresponding three-dimensional voltabsorptogram can be modelled by

$$r_{IJK} = x_{IN} y_{JN} z_{KN} + e_{IJK} \tag{17}$$

where r_{IJK} represents a three-dimensional array of data, which in voltabsorptometry are absorbances, and e_{IJK} is the error not modelled.

Trilinearity of voltabsorptometry implies that several of the three-way analysis methods developed can be applied to this kind of data.

2.2. Trilinear decomposition method

TLD [21] can be viewed as an extension of GRAM [16], but with the advantage of using multiple samples, which leads to statistically better predictions. In order to use TLD, three-way data should follow the trilinear model described in the Eq. (17). TLD is an algorithm that uses an eigenvalue decomposition to calculate x_{In}, y_{Jn} and z_{Kn}, which are vectors that describe the spectroscopic, voltammetric and relative sample composition behaviour for each pure component. If the trilinearity condition is fulfilled, then a unique solution to the decomposition problem is achieved. A detailed description of this method can be found in Ref. [21].

An improved TLD algorithm was developed [22] that differs from the original algorithm in the estimation of the intrinsic physical profiles. This algorithm avoids the problem of mismatched profiles and permits reliable structures of species concentration in the presence of complex eigensolutions. The eigenvectors and eigenvalues of the two pseudosamples generated to obtain the intrinsic profiles in each order are calculated by the NIPALS algorithm.

The method is non-iterative and decomposes the data array, which consists of second-order data matrices for standard and unknown samples, into its intrinsic factors in such a way that the estimated physical profiles are obtained. The analysis of x_{IN}, y_{JN} and z_{KN} provides a great quantity of information about the sample under study, allowing for identification of some of the components of a complex sample, for determination of that or those factors related to the analyte of interest (whose concentration is then calculated by least squares), etc.

2.3. Second-order standard addition method

The standard addition method [31], SAM, was developed to overcome the difficulties presented by the determination of an analyte in a complex sample. SOSAM [28] is an extension of SAM to second-order data and, although second order instruments provide the ‘second-order advantage’, since if the interferent changes the analyte signal, the standard addition method must be employed to ensure accurate results.

In a first step, SOSAM consists of applying TLD method on the three-dimensional array of data, in order to estimate the intrinsic factors x_{IN}, y_{JN} and z_{KN}. Each column of these matrices corresponds to one compound of the sample, and the rows of z_{KN} are its relative concentration in the samples. Next, once the **Z** scores have been calculated, a regression analysis of the concentration added against the predicted relative concentration is made and, finally, the concentration of the first sample is calculated as in a classical univariate standard addition analysis.

The last step, the calculation of the concentration, implies that the factor or factors related to the analyte of interest have been identified. In addition, if there

are several possible factors, 'the concentration of the analyte is the smallest estimate that does not change when successive standard addition samples are included in the second-order data prior to decomposition' [28].

2.4. Capability of discrimination

Given a nominal concentration c_0, the minimum discriminable net concentration, δ, is defined as the minimum concentration of analyte that can be distinguished from the nominal with pre-fixed probability $1-\beta$.

This definition generalizes that of the detection limit since for the detection limit, the nominal concentration is zero and the hypothesis test is unilateral, whereas the discrimination capability can be applied to any nominal concentration and the statistical test is bilateral.

This parameter is determined by means of a linear regression of the **Z** scores obtained by TLD vs. the true concentrations of the analyte of interest. Next, δ is calculated in order to have

$$\alpha = \mathrm{pr}\{\,|z - z_0| > z_c/c = c_{\mathrm{nominal}}\}$$

$$\beta = \mathrm{pr}\{\,|z - z_0| \le z_c/|c - c_0| > \delta\}$$

where z is the value of the **Z** scores and z_0 is the value that corresponds to c_0 in the regression. The details of this procedure can be found in Ref. [27].

3. Experimental

3.1. Equipment

Amel System-500 and CH Instruments Model 660 were used for spectroelectrochemical measurements. A Hewlett-Packard 8452A diode array spectrophotometer was coupled to the Amel electrochemical analyzer in order to record the voltabsorptometric response in the UV–VIS range. The spectroelectrochemical three-electrode cell, manufactured at the University of Camerino, is analogous to that described earlier [32,33]. The cell was constructed cylindrically along the optical path and was equipped with optical quartz windows, using a relatively thin layer (ca. 4 mm) of supporting electrolyte. The thin-layer electrode was a conventional platinum minigrid electrode. The three-electrode system was completed by means of a platinum auxiliary electrode and a saturated (KCl) Ag/AgCl reference electrode.

3.2. Chemicals

All chemicals were of reagent grade and were used without further purification. All the solutions were prepared from water, which was doubly distilled and subsequently deionized. The spectroelectrochemical measurements were carried out in a 0.5 M CH_3COOH and 1 M $HClO_4$ supporting electrolyte medium.

Two sets of samples were used. Set A: eight calibration samples containing *o*-tolidine in the range 3.2×10^{-5}–2.56×10^{-4} M were prepared, potassium ferrocyanide being added in identical concentrations to all eight samples (3.0×10^{-3} M). Set B: six additions of *o*-tolidine standard solution have been made to a sample that contained *o*-tolidine 8.8×10^{-5} M and potassium ferrocyanide 3.0×10^{-3} M, the procedure being replicated two times.

3.3. Procedure

Each sample was deoxygenated with argon for 10 min and next placed in the cell. A cyclic voltammetric curve was recorded in the potential range 0.3–0.75 V (scan rate = 0.001 V s^{-1}) for each sample, the spectra being taken every 2 nm in the spectral range 350–560 nm at uniform intervals of time during the potential scans.

4. Results and discussion

4.1. Determination of the capability of detection

The determination of the capability of detection of a univariate analytical method has been well established by the International Organization for Standardization (ISO). In the ISO 11843-1 [2], the minimum detectable net concentration is defined as 'the true net concentration of the analyte in the material to be analysed which will lead, with probability $1-\beta$ to the correct conclusion that the concentration in the analysed material is different from a

blank material'. Thus, the ISO has proposed a methodology that considers the probabilities of errors α (false positive) and β (false negative) [3]. The method consists of determining the presence or absence of an analyte through a hypothesis test, which allows one to decide between the null hypothesis 'there is no analyte' and the alternative hypothesis 'there is analyte', evaluating the probabilities of errors α and β. In addition, the method leads to the establishment of the 'capability of detection curves' of an analytical procedure, which are the operating characteristic curves of the decision rule and are expressed as a hypothesis test in terms of concentration through a calibration function. This method has been implemented in the program DETARCHI [34], which determines both the capability of detection and the capability of detection curves of the analytical procedure.

The method is based on performing a classical calibration regression that requires univariate and specific signals, i.e. this methodology has been developed to determine the capability of detection of zero-order instruments [6]. However, this methodology cannot be used with analytical procedures that provide a vector or a matrix of analytical responses for each sample. So, the determination of the capability of detection is not well established when the calibration model has to be calculated by multivariate soft-modelling techniques or when it may depend on the sample matrix or even on the actual concentration of the analyte if the signal–concentration relationship is not linear.

4.1.1. Trilinear decomposition of voltabsorptometric data

Despite the great potentiality of second-order techniques (e.g. spectroelectrochemistry, chromatography–spectroscopy, emission–excitation fluorescence) the direct application of the method proposed by the ISO to second-order data is not possible. A suitable approach proposed in this paper to calculate the capability of detection in this case consists of carrying out the decomposition of the three-way data by means of the TLD, calculating the relative concentrations obtained for the analyte of interest and relating them to the real concentrations by means of a univariate regression analysis. Next, the regression model obtained, adequately validated, is used to determinate the capability of detection of the analytical method in a similar way as ISO indicates. Thus, the interpretability of zero-order calibration is maintained, as Faber et al. [4] have suggested for a successful framework of analytical figures of merit for second-order calibration.

Besides this, since the proposed methodology implies the use of the TLD method of analysis that has the second-order advantage, it would also be possible to perform the analysis in the presence of unknown or uncalibrated interferents. This allows for the determination of the capability of detection of a second-order technique just in the conditions of the chemical analysis, even if the analysis has been carried out in a complex matrix. In this way, the capability of detection can be determined in the presence of interferents and independently of their concentrations since TLD extracts the net signal corresponding to the analyte of interest, separating its underlying contribution to the signal from the rest of the contributions. So, the proposed method may be applied to second-order data only if its three-way structure can be decomposed by means of TLD.

In order to show the application of the proposed procedure to a second-order technique of analysis, spectroelectrochemistry has been analysed. The trilinear nature of the spectroelectrochemical data allowed us to consider that the TLD method could be a suitable tool to decompose the voltabsorptometric data and to obtain the relative concentrations (**Z** scores) related to the analyte of interest. Next, once these values are correctly selected, they are used in the univariate regression analysis to determine the capability of detection.

This figure of merit has been calculated for the thin-layer cyclic voltabsorptometric determination of a very common compound in the spectroelectrochemical reports, *o*-tolidine. Initially, *o*-tolidine undergoes an oxidation at a platinum minigrid electrode giving the oxidized form, which provides the spectral signal in the UV–VIS range, and next, a reducing step is performed. Fig. 1a shows the cyclic voltabsorptometric signal corresponding to the described redox process.

In order to show the 'second-order advantage' of the mathematical tool used, an interferent has been introduced in the sample, potassium ferrocyanide, which overlaps both the electrochemical and the spectroscopic signals of *o*-tolidine, as can be seen in Fig. 1b. In addition, this kind of common interference could lead to high errors when the capability of

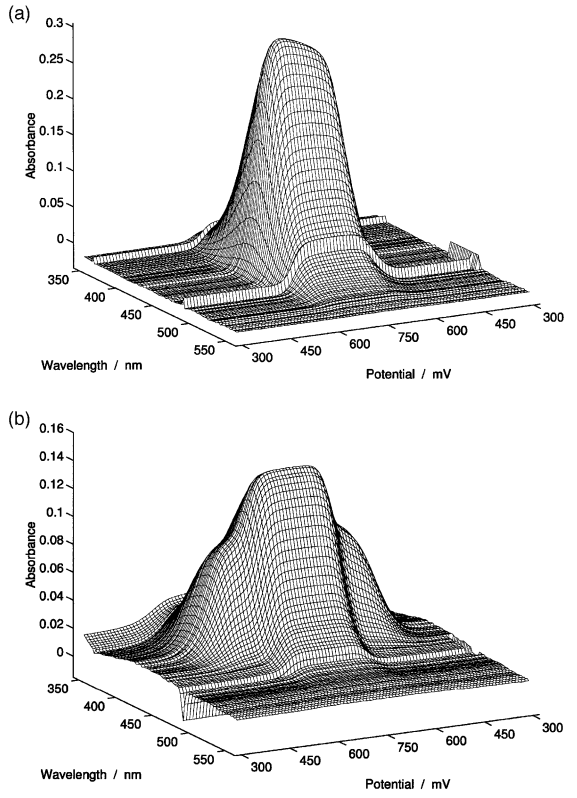

Fig. 1. Second-order instrumental response of (a) *o*-tolidine 2.0×10^{-4} M and (b) *o*-tolidine 3.2×10^{-5} M and potassium ferrocyanide 3.0×10^{-3} M.

detection is determined in the univariate classical way, taking into account only the peak absorbance or the peak current for the analyte of interest if the analysis is carried out by UV–VIS or cyclic voltammetry, respectively.

The first step in the proposed methodology consisted of the application of TLD method to the three-way data, those of set A. The concentration of the samples was near to the minimum detectable value of concentration as the determination of the capability of detection requires. Furthermore, the samples contained a relatively high concentration of potassium ferrocyanide.

These samples were measured following the experimental procedure described in the Experimental section. The data matrices obtained at the different concentrations of *o*-tolidine were organized to form a three-way data set of size $91 \times 106 \times 8$, where the first value refers to the potentials in each cyclic voltammogram, the second is the number of wavelengths in each spectrum, and the third is the number of spectroelectrochemical runs recorded. Next, the trilinear decomposition of the cube of data was made applying a home-made *m*-function implemented on MATLAB [35], developed on the base of the improved TLD algorithm [22].

Following the criterion indicated in Section 2.3, referring to the incorporation of successive samples to evaluate the changes in the trilinear decomposition, the best solution was that corresponding to the decomposition carried out with three factors. The intrinsic physical profiles estimated by TLD for them are shown in Figs. 2, 3 and 4, whereas Fig. 5 shows the estimated **Z** scores. Fig. 5 makes obvious that only the scores corresponding to the first factor were correlated to the *o*-tolidine concentration, which indicates that this was the factor related to the analyte of interest. To confirm this, the intrinsic physical profiles corresponding to this factor are analysed and compared with the spectrum and voltammogram of the chemical specie (Fig. 2). The comparison of one of the profiles with the spectrum is not problematic since both the spectrum and the profile are estimated from absorbances, and it is just enough with scaling the data. However, for comparing the other profile to the voltammogram, it should be taken into account that the profile is calculated from absorbances and the voltammogram is the current recorded, so the shape of the signals has to be necessarily different since the first has no capacitive contribution to the response. The parameters that can lead to identify the compound related to the profile are the oxidation and reduction peak potentials. However, more or less pronounced differences can be found between the values estimated from the voltammetric profile and the experimental values since a distortion of the experimental voltammogram due to the absence of capacitive contributions is expected in the estimation of the profile. In the case of the first factor, both spectroscopic and voltammetric (peak potentials) profiles are clearly related to *o*-tolidine, which corroborates the conclusion drawn from Fig. 5.

On the other hand, second and third factors (Figs. 3 and 4) are related to other components of the matrix of the sample not related to *o*-tolidine that do not vary

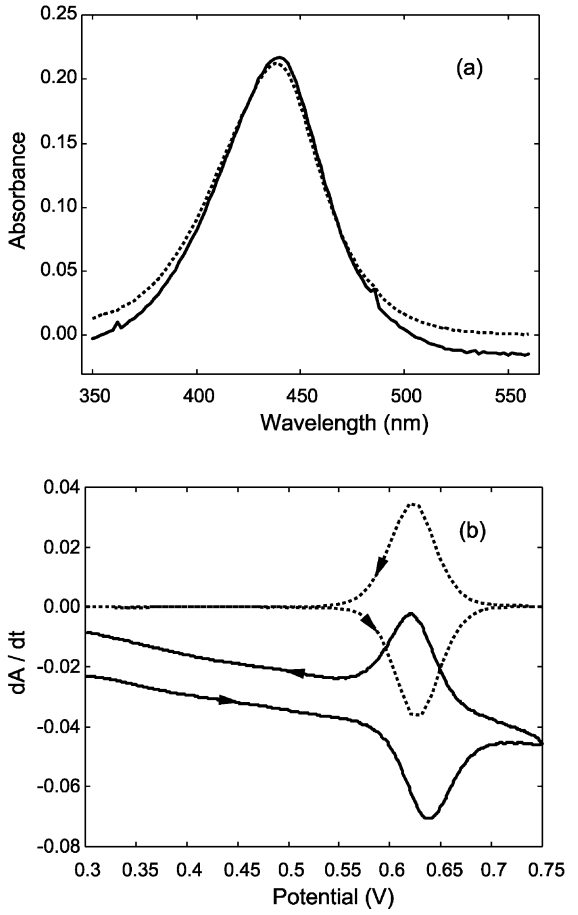

Fig. 2. Dotted line: estimated spectroscopic (a) and voltammetric profile (b) for first factor. Solid line: normalized true spectrum (a) and cyclic voltammogram (b) of *o*-tolidine 3.2×10^{-5} M (the ordinates axis for solid line (b) is the normalized current, in amperes).

during the analysis and to sources of experimental variability. In the decomposition step, the TLD method is capable to discriminate the contribution to the signal of *o*-tolidine (factor 1) from the rest of contributions, whichever their origin is.

4.1.2. Univariate linear regression and outlier detection

Next, the procedure involves the determination of a linear relation between the analyte concentration of the calibration samples and the estimated **Z** scores. This relation is established by means of the least squares (LS) regression since it provides the most probable parameters for the model, and the estimators obtained are unbiased and of lesser variance. However, if some datum lies outside the linear tendency, for example, because it is experimentally erroneous, it cancels the high inferential capability of the LS causing an error in the values of the slope and intercept.

Because of this, the procedure proposed comprises in a first step the application of the least median squares (LMS) regression [36] that has been used in the calculation of linear ranges and capabilities of detection as a method for detecting and foreseeing the presence of outliers [37,38,39]. This regression is implemented in the program PROGRESS [36] that provides an objective criterion to detect outliers. Next,

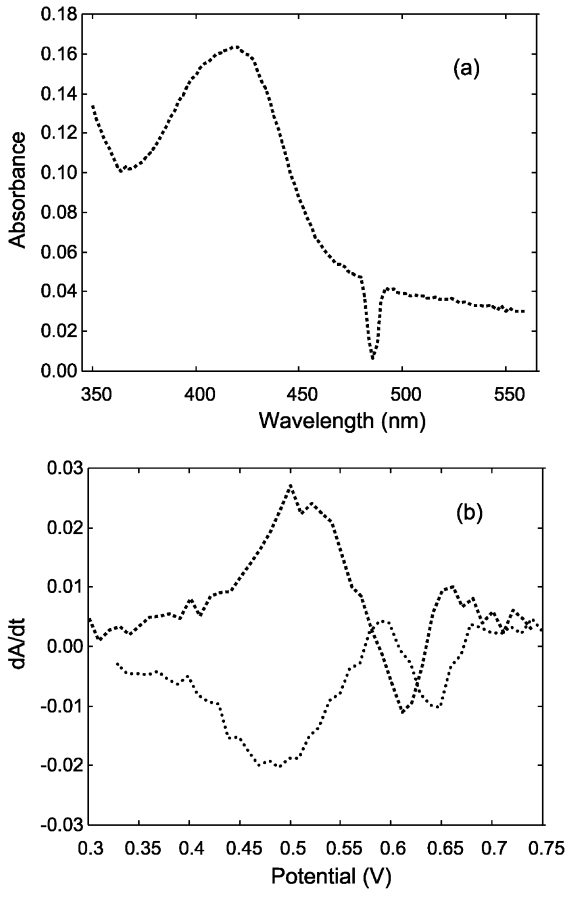

Fig. 3. Estimated spectroscopic (a) and voltammetric profile (b) for second factor.

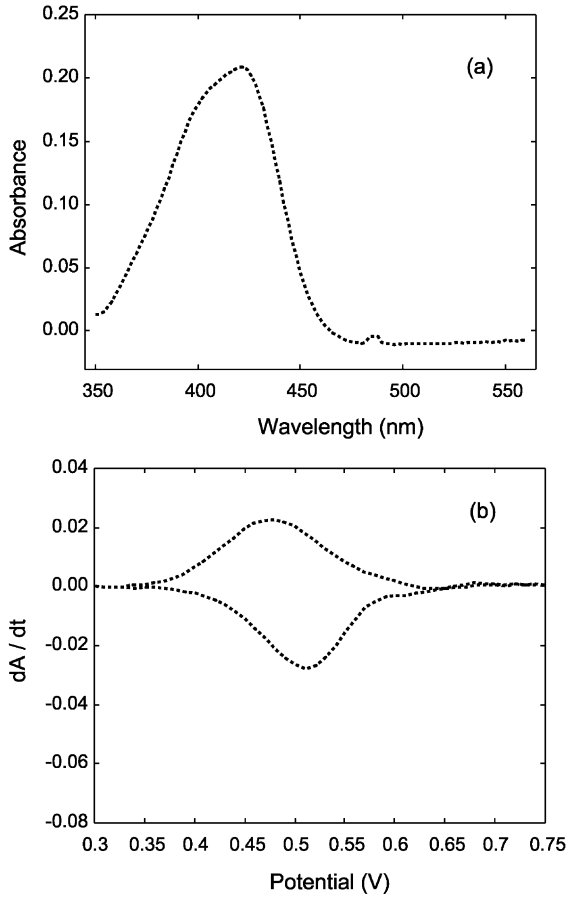

Fig. 4. Estimated spectroscopic (a) and voltammetric profile (b) for third factor.

the outliers are eliminated from the calibration set, doing the LS regression with the remaining data. This regression, the so-called 'reweighted least squares regression', means the final relation between concentration and **Z** scores.

4.1.3. Capability of detection and capability of detection curves

The final step of the procedure consists of calculating the capability of detection as ISO 11843 indicates for univariate analytical procedures, but taking as ordinates the **Z** scores estimated in the first step. To calculate the capability of detection, the program DETARCHI [34] has been used. This program implements the Clayton method [40] and provides the capability of detection curves.

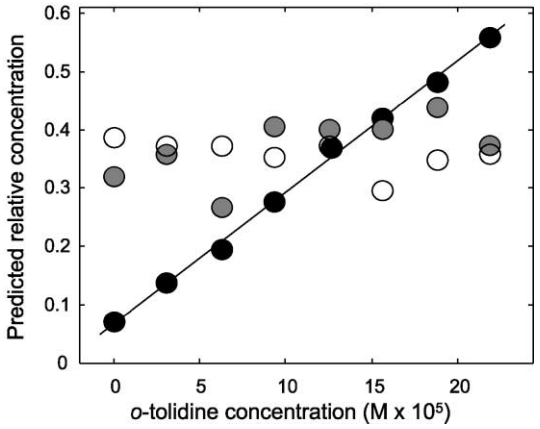

Fig. 5. **Z** scores for first (black), second (grey) and third (white) factors.

The values of the capability of detection obtained for several values of α and β are shown in Table 1, where it can be seen that the lowest concentration measured corresponds to α and β close to 2.5%. Likewise, Fig. 6 shows the capability of detection curves obtained for the second-order method studied. It is clear that the capability of detection of an analytical method of analysis is not a definite value but it depends on α and β that the analyst can assume and on the future replicates on test samples.

4.2. Determination of the capability of discrimination

The **Z** scores estimated in Section 4.1.1 have been used here to determine the capability of discrimination following the procedure indicated in Section 2.4. The result of this analysis for $\alpha = 0.05$ is shown in Fig. 7. Each curve is associated with a value of β in such a way that, e.g. for $c_{\text{nominal}} = 1.5 \times 10^{-4}$ M of *o*-tolidine, a concentration of 0.065×10^{-4} M can be discriminated with a probability $\beta = 0.5$. If one wants to assume the probability of incorrectly accepting that a test sample is 1.5×10^{-4} M, the minimum discrim-

Table 1
Capability of detection for different values of α and β

α	β	Capability of detection (M)
0.010	0.010	4.23×10^{-5}
0.025	0.025	3.25×10^{-5}
0.050	0.050	2.56×10^{-5}

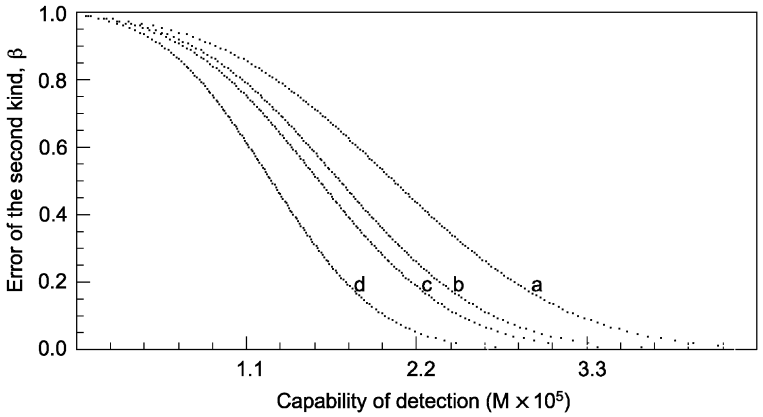

Fig. 6. Capability of detection curves for $\alpha = 0.01$. Each curve corresponds to (a) 1, (b) 2, (c) 3 and (d) infinite replicates.

inable concentration increases, e.g. for $\beta = 0.05$, the δ value is 0.130×10^{-4} M.

In addition, to make it easier to read of the figure, some straight lines have been drawn that correspond to the minimum discriminable concentration equal to 5%, 10% and 15% of the nominal value. It is clear that the analysed procedure does not allow one to discriminate 5% of the nominal concentration in the studied range with probability β lower than 0.1. However it is possible to discriminate 10% of the nominal concentration with probability $\beta = 0.05$ if $c_{\text{nominal}} > 1.3\ 10^{-4}$ M.

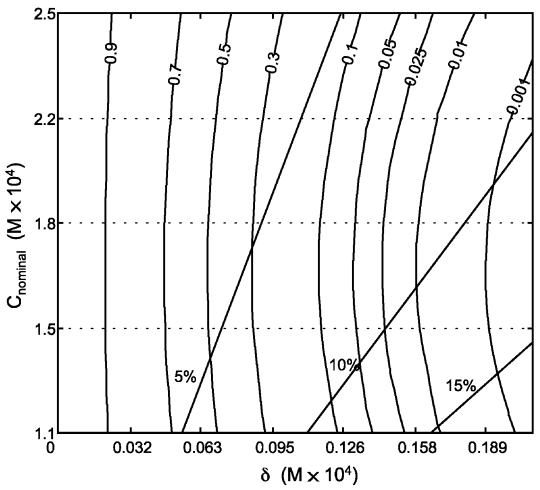

Fig. 7. Capability of discrimination curves. The nominal concentrations are in ordinates and δ values in abscissas. The value of β associated with each curve is indicated in the plot. The straight lines represent the corresponding percentages of nominal concentration that can be discriminated.

4.3. Second-order standard addition on real samples

As it has been previously indicated, if the presence of an interferent changes the analyte signal in a given matrix, e.g. overlapping the analyte response, the standard addition method must be used in order to ensure accurate results. In this part of the paper, the same chemical example used in the pervious section to evaluate the applicability of the method proposed to spectroelectrochemical data has been analysed. Thus, SOSAM has been applied to calculate the concentration of *o*-tolidine in samples that contained potassium ferrocyanide in sufficiently high concentration to seriously overlap the *o*-tolidine signal, which would enormously interfere in a classical analysis.

To carry out the analysis, the set B described in the Experimental section was used. In a first step, the analysis of the data was carried out by means of the classical univariate standard addition method applied separately in each domain. This implied considering in the determination the maximum of the spectroscopic peak when no oxidation occurred, whereas in the voltammetric determination, the peak current graphically measured on the oxidation wave was taken. Thus, the analysis has been performed by selecting the absorbance at 438 nm in the spectroscopic domain and the current peak at 0.621 V in the electrochemical

domain. As in the previous case, LMS regression has been used to detect and eliminate the possible outliers before applying the LS regression. The analysis has also been carried out to the samples of set A, considering that seven additions of *o*-tolidine standard solution were made on a sample that contained *o*-tolidine 3.27×10^{-5} M and potassium ferrocyanide 3.0×10^{-3} M.

The relative errors obtained using the univariate standard addition method are shown in Table 2. It can be seen that, in some cases, values of up to 50% or higher have been reached although the correlation coefficients of the models are good. Since the possible outliers have been removed, the high level of errors in the analysis must be attributed to the interference of the matrix in the determination, i.e. the presence of potassium ferrocyanide that cannot be detected due to the use of a univariate signal. Regressions are good, but not the calculated concentration for the samples. This evidences the fact that when a zero-order signal is used, the required selectivity in the calibration analysis is full. Even in the case of first-order calibrations, the selectivity of the analyte signal is required, so the problem posed here has no solution using any of these calibration tools. It is necessary to use three-way methods to overcome the problem of the presence of an interferent.

So, the three-way data array has been analysed by means of SOSAM, following the procedure indicated in Section 2.3. The analysis has been carried separately with the two data sets that have been studied. In the first step of the procedure, the trilinear decomposition for the calibration set with higher concentration levels has led to three factors very similar to those obtained in the decomposition carried out in the previous section for the lower concentrations. Next, LS regressions have been made (using previously LMS to detect outliners) with the **Z** scores estimated vs. concentration of *o*-tolidine added in each case, and the concentration of the first sample of each data set has been determined as in the usual univariate standard addition method. The results are shown in Table 2. The relative errors improve enormously and are acceptable when the three-way method is used. It is clear that the use of SOSAM overcomes the interference of potassium ferrocyanide and provides successful results.

5. Conclusions

It has been demonstrated that spectroelectrochemistry is suitable for generating three-way data following a trilinear model. The decomposition of the spectroelectrochemical data may be carried out by means of the TLD algorithm and the **Z** scores determinated in this way could be used to evaluate the capability of detection of a voltabsorptometric method in the presence of interferents as well as the capability of discrimination. In addition, SOSAM has been successfully applied to determine the concentration of *o*-tolidine in the presence of a highly interferent analyte in the analysis, potassium ferrocyanide, with much fewer relative errors than the classical methodologies for the same experimental data.

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Table 2
Relative errors (%) of the standard addition method, both for univariate and multivariate analysis

Replicate	$C_{o\text{-tolidine}}$ (M)	Univariate analysis		SOSAM
		Spectrum	Voltammogram	
1	8.8×10^{-5}	22.26 (0.997)	47.41 (0.970)	6.00 (0.999)
2	8.8×10^{-5}	51.80 (0.987)	18.80 (0.999)	0.50 (0.999)
1	3.2×10^{-5}	29.55 (0.999)	− 86.00 (0.988)	− 0.90 (0.996)

The correlation coefficients of the regression analysis are shown in parentheses.

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