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Quantitative determination of tocopherols in edible vegetable oils using electrochemical ultra-microsensors combined with chemometric tools

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ABSTRACT

We have developed an electroanalytical method to quantify different isomers of tocopherols in edible vegetable oils. The method uses the square wave voltammetry on a carbon fiber disk ultramicroelectrode in benzene/ethanol + 0.1 mol L^{-1} H_2SO_4.

Because the oxidation peaks of these natural antioxidants show an important overlapping, we have used two chemometric tools to obtain the multivariate calibration model. One method was the multivariate curve resolution–alternating least square (MCR–ALS), which assumes a linear behavior, i.e., the total signal is the sum of individual signals of components, and another nonlinear method such as artificial neuronal networks (ANNs).

From the accuracy and precision analysis between nominal and estimated concentrations by both methods, we could infer that the ANNs method was a good model to quantify tocopherols in edible oil samples. Recovery percentages were between 94% and 99%. In addition, we found a difference of 1.4–6.8% between the total content of tocopherols in edible oil samples and the vitamin E content declared by the manufacturers.

1. Introduction

Vitamin E is the general term used to describe a group of eight natural isomers, which along with vitamins A, D, and K are the group of fat soluble vitamins. The eight isomers are divided into two groups: four tocopherols and four tocotrienols, which differ in saturation of the side chain. Thus, tocopherols have a saturated chain and tocotrienols an unsaturated chain with double bonds at carbons 3, 7, and 11. Within each group, isomers differ in the number and the position of methyl groups on the aromatic ring, known as α, β, γ, and δ [1].

The interest in tocopherols has increased in recent years mainly due to their ability to protect cell membranes, preventing their oxidation by free radicals, and their roles against age-related diseases, cardiovascular disorders or Alzheimer's [1].

Tocopherols are essential compounds, i.e., the body cannot synthesize. Thus, they must be supplied through the diet in small amounts. Vegetable oils are an important source of tocopherols [1]. They have a high nutritional value, and also help to prevent oxidation of lipids, which would result in the formation of undesirable compounds producing oil deterioration [2].

Different methods related to the analytical determination of tocopherols in edible oils have been described in literature [3], most of which require a preliminary stage of extraction combined with separation techniques, being the most used the HPLC chromatography [4,5]. In addition, studies have been conducted in recent years to the development of analytical procedures to allow the characterization and authentication of oils through the tocopherol total content analysis, and the differentiation and determination of their different isomers [1,6,7].

On the other hand, electroanalytical techniques have demonstrated to be reliable and fast tools to determine synthetic antioxidants in edible oils [8–10].

In addition, the application of powerful chemometric techniques helps to resolve a mixture of voltammetric signals which

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appear at close potentials, such as occur with tocopherols [11]. The chemometric techniques most used are the multivariate calibration based on different regression methods, such as multivariate curve resolution–alternating least square (MCR–ALS) [12], multi linear regression (MLR) [13,14], principal component regression (PCR) [14,15], partial least square regression (PLS) [14,16–18], and artificial neural networks (ANNs) [14,19–22].

The main advantage of chemometric techniques is their ability to obtain quantitative information from overlapping signals through mathematical procedures, without requiring any prior pretreatment of the sample.

In this work, we study the electrochemical oxidation of three tocopherol isomers (α, γ, and δ) at a carbon fiber (CF) disk ultramicroelectrode (UME) in benzene/ethanol (Bz/EtOH, 2:1)+ 0.1 mol L^{-1} H_2SO_4 by square wave voltammetry (SWV), whose anodic voltammetric signals are overlapped. Therefore, we applied two chemometric methodologies in order to obtain a multivariate calibration model: one in which the resultant signal is linear corresponding to the additive sum of the individual components by applying the MCR–ALS method and another no linear by applying ANNs.

2. Experimental

2.1. Reagents

α, γ, and δ tocopherols were obtained from Sigma Chemical Company. Benzene (Bz), and acetone (Ac) were Sintorgan, HPLC grade. Ethanol (EtOH), and H_2SO_4 were Merck p.a. All reagents were used as received. Edible oils were purchased from local supermarkets.

Commercial tocopherols and oils were dissolved in Bz/EtOH (1:2)+ 0.1 mol L^{-1} H_2SO_4 for performing their analytical determinations.

2.2. Apparatus and experimental measurements

A two compartment Pyrex cell using a conventional three-electrode configuration was used to perform SWV experiments, which was coupled to an AutoLab PGSTAT 12 potentiostat (Eco-Chemie, The Netherland). The characteristic parameters of SW voltammograms were: square wave amplitude, $\Delta E_{SW}=0.050$ V, the staircase step height, $\Delta E_s=0.005$ V, and the frequency, $f=25$ Hz.

A carbon fiber disk UME (diameter, $\Phi=11$ μm, BAS Electroanalytical System, USA) was used as the working electrode. It was pretreated as previously described [10]. The reference electrode was an aqueous saturated calomel electrode (SCE), and the counter electrode was a Pt foil of large area ($A \approx 2$ cm^2).

2.3. Software's

The neural network toolbox from the software suite MALAB 7.8 [23] was used for applications of ANNs.

MCR–ALS calculations, initial estimations, and figures of merit were calculated using an algorithm written in MATLAB, which is available in the literature [24]. An algorithm also written in MATLAB was used for generation of the ellipses [25].

2.4. Preparation of standards

2.4.1. Calibration set

The calibration set used in ANNs was obtained from 30 samples, 23 of which were ternary mixtures obtained from a central composite experimental design, rotatable and orthogonal, including nine

Table 1
Values of characteristic voltammetric signal parameters (A, D, I_p, and E_{rpp},) used as input data, and concentration values of α, γ, and δ tocopherols in Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4 used as output data in the calibration set.

Sample	$c^*_{\alpha\text{-tocopherol}} \times 10^4$ (mol L^{-1})	$c^*_{\gamma\text{-tocopherol}} \times 10^4$ (mol L^{-1})	$c^*_{\delta\text{-tocopherol}} \times 10^4$ (mol L^{-1})	$A \times 10^8$	$D \times 10^{11}$	$I_p \times 10^{10}$ (A)	E_{rpp} (V)
1	0.6	1.3	0.675	1.0621	3.2869	3.0851	0.0615
2	0.3	2.0	1.0	1.2945	4.3683	4.0634	0.0615
3	0.6	1.3	0.675	0.8975	2.7490	2.5758	0.0308
4	0.3	0.6	0.35	0.4573	1.5872	1.2697	0.0615
5	0.9	2.0	1.0	1.4116	4.1317	4.1193	0.0462
6	0.6	1.3	0.675	0.9935	3.0743	2.8591	0.0615
7	0.6	1.3	0.675	0.9282	2.8626	2.6698	0.0615
8	0.095	1.3	0.675	0.8210	2.9818	2.6291	0.0615
9	0.6	1.3	0.675	0.9676	2.8071	2.7962	0.0615
10	0.6	1.3	0.675	0.9843	2.7621	2.8472	0.0615
11	0.6	1.3	1.2	1.2103	3.5087	3.4688	0.0923
12	0.6	0.12	0.675	0.4404	1.4438	1.0378	0.2154
13	0.6	2.5	0.675	1.4244	4.9498	4.5363	0.0462
14	1.1	1.3	0.675	1.0883	2.7574	2.8691	0.0462
15	0.9	0.6	0.35	0.6229	1.7011	1.4625	0.0154
16	0.6	1.3	0.675	1.0493	3.1416	3.0361	0.0615
17	0.3	0.6	1.0	0.6987	2.2808	2.0066	0.1385
18	0.9	0.6	1.0	0.7972	2.0222	1.9724	0.1231
19	0.6	1.3	0.675	0.9133	2.7899	2.6267	0.0769
20	0.9	2.0	0.35	1.1933	4.1337	3.6275	0.0308
21	0.3	2.0	0.35	1.1833	4.6026	3.9668	0.0308
22	0.6	1.3	0.13	0.7843	2.8496	2.4186	0.0154
23	0.6	1.3	0.675	0.9122	2.7871	2.6268	0.0615
24	0.5	0.2	0	0.2228	0.9186	0.5460	0.1231
25	0.6	2.0	0	1.0575	4.3003	3.5255	0.0154
26	0	1.0	0.8	0.7405	2.6197	2.3396	0.0923
27	0	1.2	0.2	0.6347	2.8083	2.1949	0.0308
28	0.6	0	0	0.1571	1.1117	0.5292	0.2308
29	0	1.3	0	0.6052	3.0858	2.2127	0.0154
30	0	0	0.6	0.2774	1.5649	0.9736	0.1846

A is the voltammetric signal area. D is the sum of absolute values of maximum and minimum of the first derivative of the voltammetric signal. I_p is the peak current, and E_{rpp} is the relative peak potential.

central points with five concentration levels for each substrate. Other four samples were binary mixtures, and the other three corresponded at individual solutions of each substrate (Table 1).

On the other hand, the calibration set used in MCR–ALS calculations was composed by 26 samples, 23 of which were ternary mixtures, and the other three corresponded to the individual substrates.

Concentration ranges were selected on the basis of results of linear calibration, previously obtained from univariate experiments performed for each substrate. Therefore, concentration ranges were from 9.0×10^{-6} to 1.1×10^{-4} mol L^{-1}, from 1.2×10^{-5} to 2.5×10^{-4} mol L^{-1}, and from 1.3×10^{-5} to 1.2×10^{-4} mol L^{-1} for α, γ, and δ tocopherols, respectively. Then, these standard concentrations were called as nominal concentrations.

2.4.2. Validation set

The validation set used to validate the calibration model determined by ANNs consisted of 16 solutions obtained from a central composite experimental design (see Table 2).

In all cases, SW voltammograms were recorded by triplicate using random concentrations of substrates.

2.5. Chemometric tools

2.5.1. MCR–ALS applications

First, the experimental data matrix was built, called as matrix *D*. Each row of this matrix corresponds to SW voltammograms recorded for different solutions, and each column corresponds to current values measured at the corresponding potential. Then, the MCR method was applied to the matrix *D* for resolving responses of pure substrates. The MCR method is based on a bilinear model, which relates concentrations and voltammetric responses of pure substrates, assuming that individual responses are additive [26].

In matrix algebra, the matrix *D* is expressed by the following equation:

$$D = CV^T + E \quad (1)$$

where $D(i,j)$ dimensions are i samples (voltammograms) per j potentials; $C(i,k)$ is the concentration matrix of different k substrates present in the sample, and $V^T(k,j)$ is the transpose matrix, whose k rows contain voltammograms of pure substrates, and $E(i,j)$ is the residual matrix. Therefore, the MCR method allows estimating C and V^T matrixes using a classical scheme of the alternating least squares (ALS) procedure. For starting the ALS iteration procedure is necessary to have an initial estimation of voltammograms of each individual substrate. We used an algorithm that relies on the determination of pure variables for the initial estimation [27].

If the initial estimation is composed by voltammograms of each substrate, the solution for the concentration profiles are calculated using the following equation:

$$C = D(V^T)^+ \quad (2)$$

where $(V^T)^+$ is the pseudo-inverse of V^T.

On the other hand, in the optimization step, which involves an iterative cycle, we used a series of restrictions for obtaining solutions with physical meaning [27]. Restrictions were: (a) non-negativity, which prevents the presence of negative values in concentration profiles and voltammograms, and (b) unimodality, which ensures the presence of a single peak in voltammograms.

Finally, values calculated at the optimization step from the calibration set were correlated with nominal concentrations, obtaining the corresponding linear regressions and root mean square errors (*rmse*) for each substrate (see Section 2.5.3).

2.5.2. Applications of ANNs

The objective of this study was to predict tocopherol concentrations in oil samples according to SW voltammograms recorded. The network used in the simulation of concentrations was the multilayer perceptrom network with the backpropagation supervised learning method. These networks are suitable for solving problems like ours, given its ability to generalize, and no mathematical knowledge is required on the function relating the input patterns with output patterns [28–30].

Different architectures were compared to build and validate the predictive model of network, which consisted of an input layer, one hidden and one output. The number of neurons of the input layer was equal to the number of independent variables entered into the model, in this case the four parameters obtained from voltammetric responses (see Section 2.5.2.1), and the number of neurons in the output layer corresponded to the number of model output variables, i.e., concentrations of α, γ and δ tocopherols.

On the other hand, the number of neurons in the hidden layer was obtained from the best architecture of ANNs through the following procedure: (1) An ANNs with a number N of neurons in the hidden layer were created. (2) The type of training and the transfer function were defined. (3) The network was trained with the calibration data set, considering a 70% of learning, a 15% for monitoring, and another 15% to test the network. (4) The network was validated using the validation data set (independent of

Table 2
Concentrations of α, γ, and δ tocopherols in Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4 used in the validation step. Normal and bold concentration values are nominal and estimated concentrations, respectively.

Sample number	$c^*_{\alpha\text{-tocopherol}}\times10^4$ (mol L^{-1})		Percentage relative errors	$c^*_{\gamma\text{-tocopherol}}\times10^4$ (mol L^{-1})		Percentage relative errors	$c^*_{\delta\text{-tocopherol}}\times10^4$ (mol L^{-1})		Percentage relative errors
1	0.5	**0.48**	2.3	2.2	**2.32**	5.4	0.5	**0.52**	5.8
2	1.2	**1.24**	3.6	0.8	**0.87**	9.3	1.1	**0.99**	10
3	0.5	**0.51**	3.0	0.8	**0.78**	1.9	0.5	**0.43**	14.5
4	1.2	**1.18**	1.3	2.2	**1.98**	10	0.5	**0.54**	8.0
5	1.2	**1.21**	1.0	2.2	**1.95**	11.0	1.1	**0.94**	14.3
6	1.2	**1.24**	3.3	0.8	**0.83**	3.3	0.5	**0.46**	8.0
7	0.5	**0.51**	2.0	0.8	**0.84**	4.8	1.1	**1.0**	9.1
8	0.85	**0.86**	1.5	1.5	**1.44**	4.14	0.80	**0.72**	9.8
9	0.5	**0.47**	6	2.2	**2.14**	2.72	1.1	**1.23**	11.8
10	1.5	**1.49**	0.91	1.5	**1.52**	0.93	0.8	**0.72**	10.3
11	0.85	**0.93**	9.2	1.5	**1.54**	3.05	1.3	**1.34**	2.9
12	0.85	**0.84**	1.2	2.7	**2.49**	7.6	0.8	**0.84**	5
13	0.85	**0.80**	5.4	1.5	**1.38**	7.5	0.8	**0.83**	4.5
14	0.23	**0.25**	8.6	1.5	**1.57**	5.2	0.8	**0.69**	12.9
15	0.85	**0.86**	2	1.5	**1.53**	0.5	0.27	**0.25**	7.4
16	0.85	**0.86**	1.65	0.26	**0.243**	6.5	0.8	**0.81**	1.3

calibration data set), and (5) *rmse* values were obtained from points three and four.

The above procedure was made by combining different types of training and transfer functions. After obtaining the errors of different architectures, the best amount of the hidden layer neurons, the transfer function, and of the training type based on the least mean squared normalized error (*mse*), the relative errors (*re*), and *rmse* for three substrates, avoiding the overfitting (Section 2.5.3) were selected.

2.5.2.1. Variables involved in ANNs. For training the network is necessary to have input and output (target) data. Therefore, instead of using the full square wave voltammogram, we used only four characteristic parameters of the signal in order to reduce the dimensionality of the system based on a method proposed in the literature [31], minimizing the risk of over fit and increased robustness of the network during the learning process (Table 1). Thus, input data were the voltammetric signal area (A), the sum of absolute values of maximum and minimum of the first derivate of voltammetric signal (D), the peak current (I_p), and the peak potential (E_p) of each square wave voltammogram recorded in the potential region between 0.3 and 0.95 V. Output data were α, γ and δ tocopherol concentrations.

2.5.3. Results validation

To evaluate the quality of the results obtained using the MCR–ALS method and to compare how well experimental data of matrix D are explained, the percentage of lack-of-fit (ALS lof) was calculated through the following equation [26]:

$$lof(\%) = 100\sqrt{\frac{\sum_{ij}(d_{ij}-\hat{d}_{ij})^2}{\sum_{ij}d_{ij}^2}} \quad (3)$$

where d_{ij} are elements of the experimental matrix D, i.e., current values measured for sample i to potential j, and $\hat{d}_{ij}$ are the corresponding elements calculated by ALS.

In addition, to evaluate the quality of the quantitative predictions of concentrations obtained from the different methods, the *rmse* between nominal and estimated concentrations for each substrate, and the percentage relative error (*re*%) were calculated by applying the Eqs. (4) and (5), respectively

$$rmse = \sqrt{\frac{\sum_{i=1}^{n}(\hat{c}_i-c_i)^2}{n}} \quad (4)$$

$$re(\%) = 100\sqrt{\frac{\sum_{i=1}^{n}(\hat{c}_i-c_i)^2}{\sum_{i=1}^{n}c_i^2}} \quad (5)$$

where $\hat{c}_i$ and c_i are estimated and nominal concentrations, respectively.

In addition, a procedure to verify if the point (1,0) is contained in the confidence elliptical region of the slope and the intercept of the fitted curve between nominal and estimated concentrations was used to evaluate if those concentrations differ or nor statistically [32,33].

2.5.4. Determination of tocopherols in oils

Sunflower, corn, soybean, and two alternative varieties such as canola and grape seeds edible oils were studied. In these oils is common to find different isomers of vitamin E, such as α, γ and δ tocopherols.

For electroanalytical determinations, 1.5 mL of oil was dissolved in 15 mL of Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4. Then, 10 mL of the solution was carried out to the electrochemical cell, and the corresponding square wave voltammograms were recorded [10].

3. Results and discussion

A drawback that may occur in the application of electroanalytical techniques is their lack of selectivity.

Square wave voltammograms obtained for the electrochemical oxidation of α, γ, and δ tocopherols at a CF disk UME in Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4 are shown in Fig. 1a. As can be observed, electrochemical signals of tocopherols appear at potential regions next to each other, showing a marked overlapping. In addition, the sum of the signals of the individual tocopherols was not equal to the signal obtained from the voltammogram recorded for mixtures of tocopherols, exhibiting variable differences as a function of concentration levels. Thus, two models of multivariate calibration, MCR–ALS and ANNs, were implemented to solve overlapped SW voltammograms and quantify tocopherols in oil samples.

MCR–ALS corresponds to a linear behavior, while ANNs correspond to a nonlinear behavior between electrochemical responses and concentrations. Finally, to determine whether the experimental behavior justifies the use of a non-linear calibration model,

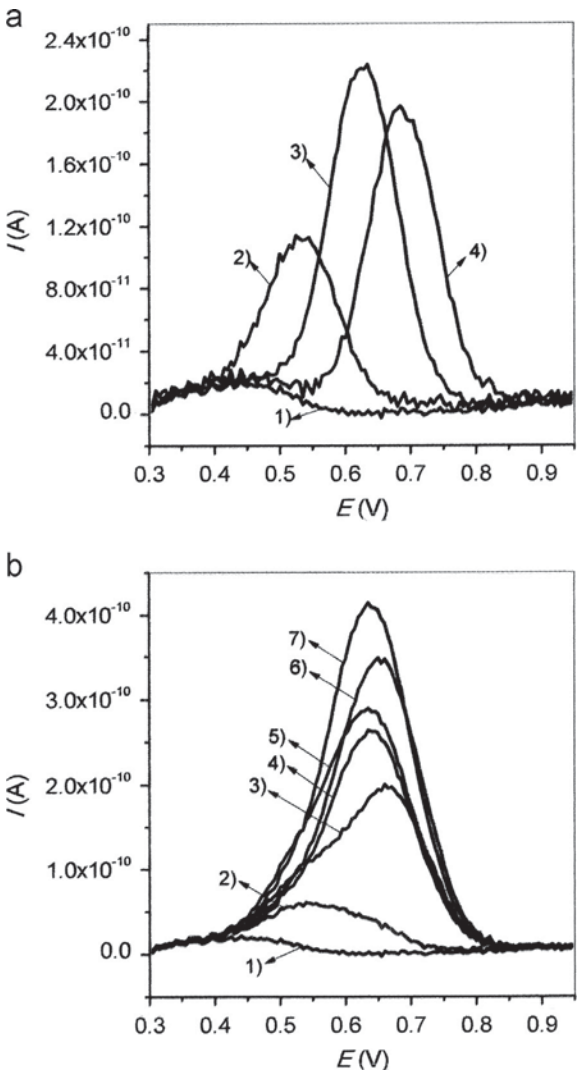

Fig. 1. (a) Square wave voltammograms recorded for: (1) the blank solution; (2) α, (3) γ, and (4) δ tocopherols at a CF disk UME (Φ=11 μm) in Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4. Tocopherol concentrations: 1.2×10^{-4} mol L^{-1}. δE_{SW}=0.050 V, δE_s=0.005 V, f=25 Hz. (b) Square wave voltammograms recorded for some mixtures of tocopherol of different concentrations used in calibration sets: (1) blank, (2) sample 5, (3) sample 11, (4) sample 14, (5) sample 23, (6) sample 18, and (7) sample 24 (see Table 1). Other experimental conditions are the same that those of Fig. 1a.

studies were conducted to estimate the accuracy and the precision between nominal concentrations and those estimated by both models.

Some of SW voltammograms recorded for 30 samples after subtracting background currents are shown in Fig. 1b. These samples correspond to different calibration sets used.

Table 1 shows the concentrations of solutions used in the calibration set, and parameters obtained after processing each voltammogram. For the MCR–ALS calibration model was used voltammograms and their respective concentrations, while for the ANNs model was used voltammogram parameters as input data and concentrations as output data (see Section 3.2).

3.1. Calibration model from MCR–ALS

To obtain the calibration model from this methodology we used the calibration set described in Section 2.4.1.

After optimization, concentration profiles (Fig. 2a) and normalized square wave voltammograms (Fig. 2b) were obtained.

The difference between the input matrix D and that produced by the product CV^T using the algorithm MCR–ALS was of 1.1% (see Section 2.5.3). A plot of estimated vs. nominal concentrations for each substrate is shown in Fig. 3. Parameters of corresponding linear regressions and respective *rmse* errors are shown in Table 3. As can be observed, good slopes and correlation coefficients for α and γ tocopherols (close to one) were found, while those corresponding to δ tocopherol showed a slightly greater deviation from the ideal slope.

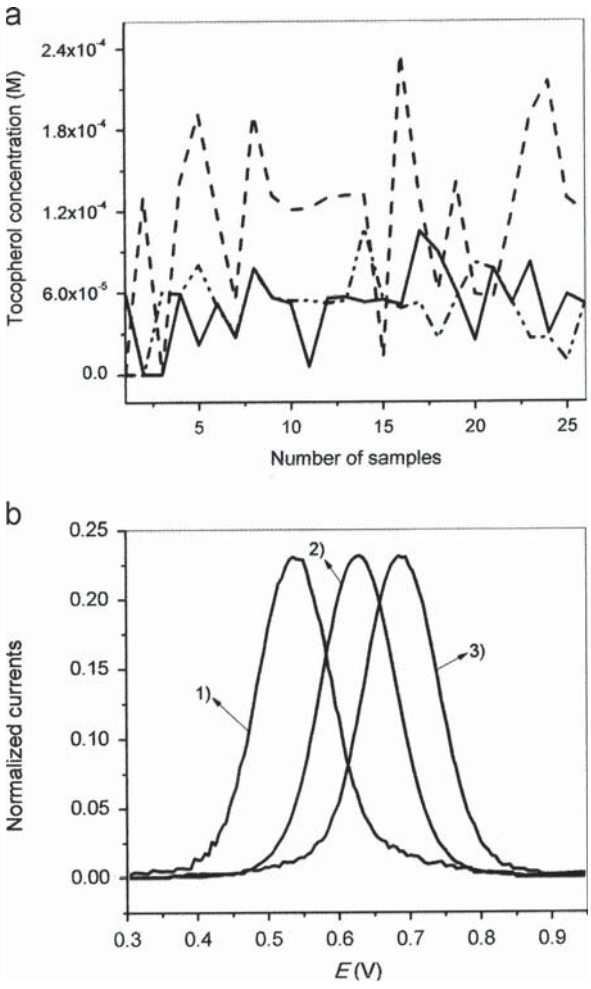

Fig. 2. (a) Optimized concentration profiles by MCR–ALS for α (solid line), γ (dash line), and δ (dash dot dot line) tocopherols. (b) Optimized square wave voltammograms by MCR–ALS for: (1) α, (2) γ, and (3) δ tocopherols.

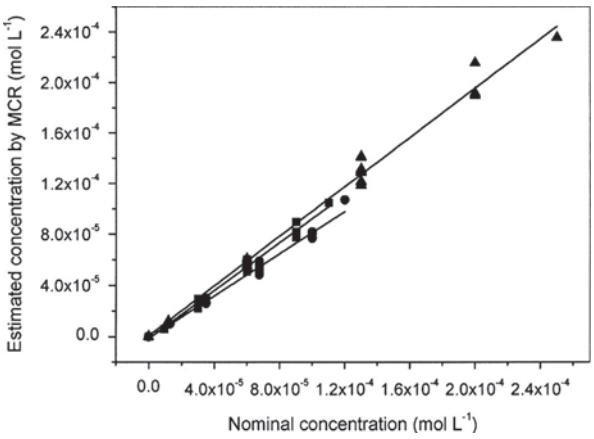

Fig. 3. Dependence between nominal and MCR–ALS calculated concentrations for each substrate. (■) α, (●) γ, and (▲) δ tocopherols.

Table 3
Linear regression parameters obtained from the dependence between estimated by MCR–ALS and nominal concentrations.

Tocopherols	Intercept (mol L^{-1})	Slope	r^*	*rmse* (mol L^{-1})
α	$(-1.6 \pm 1.5) \times 10^{-8}$	(0.94 ± 0.03)	0.9813	7.7×10^{-6}
γ	$(0.6 \pm 0.2) \times 10^{-8}$	(0.97 ± 0.03)	0.9855	13.8×10^{-6}
δ	$(1.2 \pm 1.8) \times 10^{-8}$	(0.82 ± 0.03)	0.9744	6.4×10^{-6}

* r is the linear correlation coefficient.

3.2. Calibration model from ANNs

First, we applied the methodology explained in Section 2.5.2 to find the most suitable network architecture for the resolution of measured signals.

Therefore, the best network model was obtained using a 4–4–3 architecture, i.e., four neurons in the input layer, four in the hidden layer, and three in the output layer. We used Tansig sigmoid transfer function in the hidden layer, and the Purelin linear function for the output layer. The most appropriate algorithm in the training stage was that of Levenberg–Marquardt [23].

As it was previously described, parameters obtained from each SW voltammogram were used as input data to the network, and as output data the nominal concentrations, both from the calibration set (see Section 2.4.1).

A plot of the estimated concentrations obtained by the ANNs method and nominal concentrations is shown in Fig. 4. Table 4 shows the linear regression parameters and associated errors.

Moreover, the network was validated by a set of independent solutions used in the training stage (see Section 2.5.2).

Some responses of SW voltammograms obtained for solutions of the validation set, after subtracting the corresponding blank currents, are shown in Fig. 5. In addition, nominal concentrations as well as estimated concentrations using enhanced network are shown in Table 2, with relative errors for each concentration level of tocopherols.

After obtaining values for each substrate concentration, we calculated errors% *re* and *rmse* (see Section 2.5.3). They are shown in Table 5, and as can be observed, the relative errors were in the range from 1% to 2.5%.

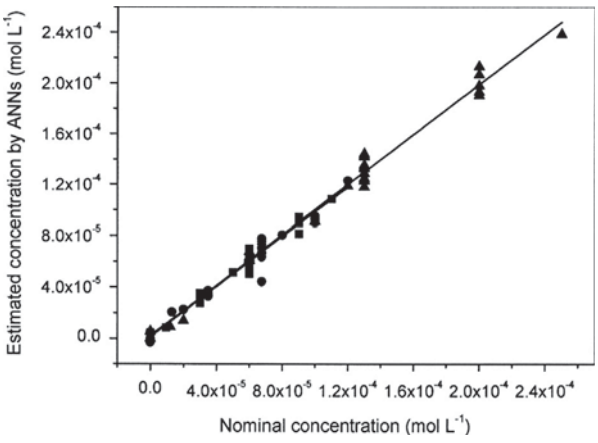

Fig. 4. Dependence between nominal and ANNs calculated concentrations for each substrate. (■) α, (●) δ, and (▲) γ tocopherols.

Table 4
Linear regression parameters obtained from the dependence between estimated by ANNs and nominal concentrations.

Tocopherols	Intercept (mol L^{-1})	Slope	r^*	*rmse* (mol L^{-1})
α	$(1.2 \pm 1.6) \times 10^{-8}$	(0.97 ± 0.03)	0.9761	4.4×10^{-6}
γ	$(1.6 \pm 2.9) \times 10^{-8}$	(0.98 ± 0.03)	0.9856	7.3×10^{-6}
δ	$(2.2 \pm 2.4) \times 10^{-8}$	(0.96 ± 0.02)	0.9593	6.4×10^{-6}

* r is the linear correlation coefficient.

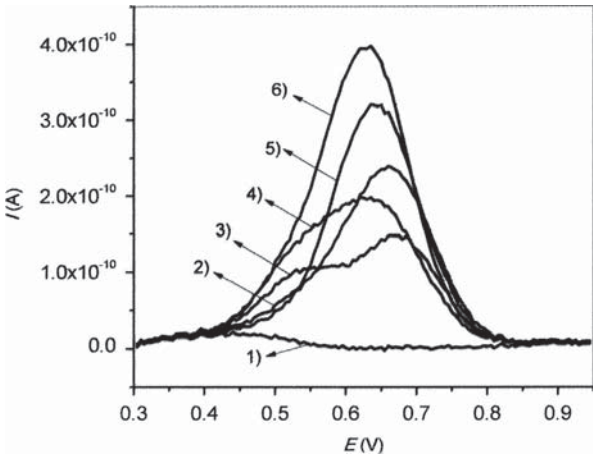

Fig. 5. Square wave voltammograms recorded for some mixtures of tocopherol of different concentrations used in the validation set: (1) blank, (2) sample 7, (3) sample 16, (4) sample 6, (5) sample 14, and (6) sample 4 (see Table 2). Other experimental conditions are the same that those of Fig. 1a.

3.3. Accuracy and precision analysis

To determine whether the concentrations estimated by methods MCR–ALS and ANNs differ or not statistically from the nominal concentrations, we examined whether the point (1,0) was included in the elliptical region of the joint confidence of slope and intercept of plots showed in Figs. 3 and 4. Therefore, plots of ellipses obtained by both methods are shown in Fig. 6 for each substrate.

As can be observed, results obtained by MCR–ALS for α and δ tocopherols have more precision than those produced by ANNs but they do not include the point (1,0), so they are less accurate (away from the ideal point, (1,0)). However, results obtained by the ANNs method include the ideal point, exhibiting a greater accuracy and a minor precision (Fig. 6a and c). It was also found that both methods had a comparable accuracy and precision for the γ-tocopherol (Fig. 6b). Thus, the method ANNs was chosen as the best for obtaining the calibration model.

Table 5
Percentual relative errors (*re*%), and root mean square errors (rmse) obtained for each tocopherol.

Tocopherols	*re* (%)	*rmse* (mol L^{-1})
α	0.89	3.0×10^{-6}
γ	1.89	0.1×10^{-6}
δ	2.5	8.0×10^{-6}

3.4. Recovery percentages

Recovery percentages were determined with corresponding standard deviations after choosing the calibration model (Table 6). The percentage recovery was calculated from six replicated measurements (six nominal, and six estimated), which were obtained from the validation set (Table 2). Thus, the samples used were 8, 11, 12, 13, 15, and 16 for the α tocopherol; 8, 10, 11, 13, 14, and 15 for γ tocopherol, and 8, 10, 12, 13, 14 and 16 for the δ tocopherol. These samples were chosen because a greater number of replicated measurements were performed for these concentration levels (0.85, 1.5 and 0.80×10^{-4} mol L^{-1} for α, γ and δ, respectively, see Table 2).

3.5. Real samples

Finally, we determined the concentration of different tocopherol isomers as well as the total content of tocopherols in different edible oil samples.

Square wave voltammograms recorded at a CF disk UME in Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4 for the different oil samples are shown in Fig. 7.

After running the algorithm, containing the optimized network using four parameters of square wave voltammograms of different oil samples, we determined the concentrations of different tocopherol isomers (Table 7). Therefore, differences between the calculated values for total content of tocopherols and those reported by the manufacturers were 2.2%, 6.8%, 1.9%, 1.4%, and 1.6% for canola, sunflower, corn, soybean, and grape seeds oils, respectively.

These results demonstrate that the application of SWV at ultramicroelectrodes combined with ANNs is a useful analytical tool for the determination of tocopherols in edible oils.

4. Conclusions

In spite of we used more samples in the methodology of artificial neural networks than in model of the multivariate calibration curves to build the calibration model, the methodology of artificial neural networks was the most appropriated to describe those responses that exhibit a deviation from linearity, as shown from analysis of the elliptical regions of confidence.

Moreover, we found that the recovery percentage in the analysis of validation samples was between 94% and 99%. In addition, we found a difference of 1.4–6.8% between the total content of tocopherols and vitamin E content declared by the manufacturers. Based on these results, we conclude that the artificial neural network trained for the

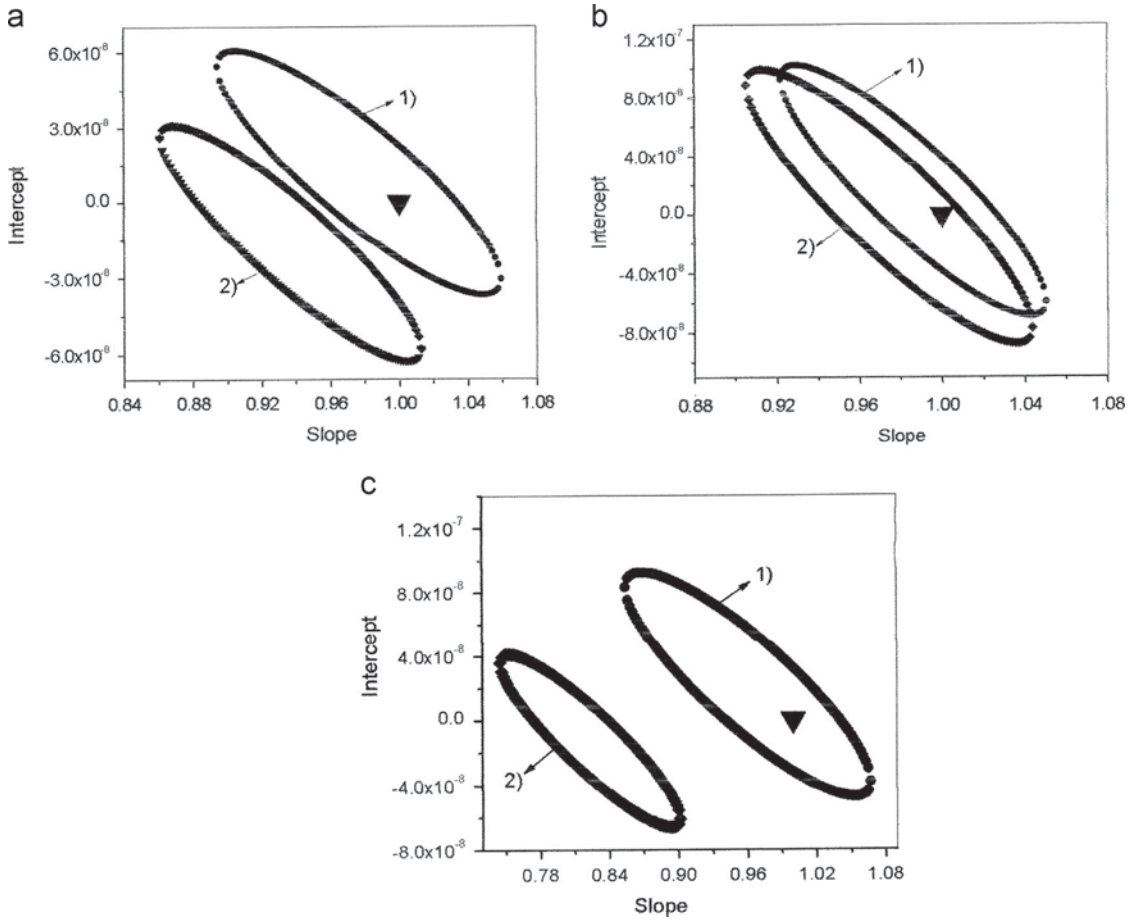

Fig. 6. Elliptical regions of the joint confidence obtained by (1) ANNs, and (2) MCR–ALS methods for (a) α, (b) γ, and (c) δ tocopherols. (▾) is the ideal point, (1,0).

Table 6
Recovery percentages obtained by the ANNs method.

Tocopherols	($c^*_{average} \pm$ standard deviation) $\times 10^4$ (mol L^{-1})	Recovery percentages
α	0.86 ± 0.04	98.8
γ	1.49 ± 0.07	99.3
δ	0.76 ± 0.07	94.7

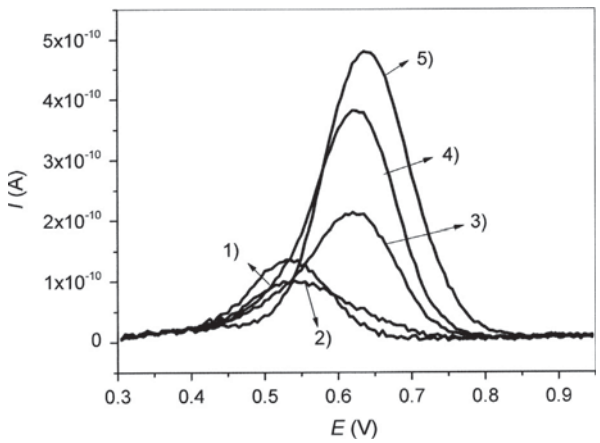

Fig. 7. Square wave voltammograms recorded for 15 mL of: (1) sunflower, (2) grape seed, (3) canola, (4) corn, and (5) soybean oils at a CF disk UME (Φ=11 μm) in Bz/EtOH (1:2)+0.1 mol L^{-1} H_2SO_4. δE_{SW}=0.050 V, δE_s=0.005 V, and f=25 Hz.

Table 7
Content of α, γ, and δ tocopherols as well as total tocopherols in edible oils.

Oil samples	$c_{\alpha\text{-tocopherol}}$ (mg L^{-1})	$c_{\gamma\text{-tocopherol}}$ (mg L^{-1})	$c_{\delta\text{-tocopherol}}$ (mg L^{-1})	$c_{total\ tocopherols}$ (mg L^{-1})	Vitamin E content declared by the manufacturer (mg L^{-1})
Canola	118.9	345.2	24.7	488.8	500
Sunflower	293.7	114.6	0	408.3	438
Corn	122.2	600.9	46.3	769.4	785
Soybean	0	614.2	283.4	897.6	910
Grape seed	279.5	55.0	0	334.5	340

calibration set is a good model for the determination of tocopherols in commercial samples of oils.

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