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Sequential injection system with higher dimensional electrochemical sensor signals

Part 1. Voltammetric e-tongue for the determination of oxidizable compounds

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Abstract

A sequential injection analysis (SIA) system was developed with the aim of obtaining an automatic and versatile way to prepare standards needed in the study of systems with higher dimensional sensor signals. To illustrate this, different analytical techniques were used in determinations of several analytes. Automated potentiometric calibrations of different potentiometric sensors, with and without interference, were carried out. Useful determinations of selectivity coefficients with two degrees of freedom were obtained. Simultaneous voltammetric determinations have also been done. Firstly, simultaneous determinations of lead and cadmium, using epoxy-graphite composite as the working electrode, have enabled a separate calibration for each metal to be obtained. Next, a voltammetric electronic tongue was designed and applied to the determination of oxidizable species. The use of artificial neural networks has solved the overlapped signal of ascorbic acid, 4-aminophenol and 4-acetamidophenol (paracetamol). A set of 63 data points was prepared automatically and has facilitated the training of an electronic tongue for these three analytes. Accurate predictions of test solutions, in the range of 12–410 μM for ascorbic acid, 17–530 μM for 4-aminophenol and 10–420 μM for paracetamol, have been achieved with RMSEs lower than 0.10 μM.

Keywords: Sequential injection analysis; Artificial neural networks; Potentiometric sensors; Voltammetric sensors; Electronic tongue

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1. Introduction

Automatic systems, such as flow injection analysis (FIA) systems, have been one of the most recurrent topics in analytical chemistry for the last two decades. The appearance of sequential injection analysis (SIA) in 1990 [1] enabled the possibility of complex sample processing schemes that FIA systems were not capable of achieving with simple manifolds. At that time, FIA was endorsed for process monitoring in analytical chemistry, where several instrumentation and methodologies could be implemented. The main disadvantages of FIA balanced the main advantages of SIA: (i) possibility of a single manifold for different analytical methods and (ii) higher robustness in manifold design.

Simple SIA systems can be designed simply by connecting a multiposition valve and a bi-directional pump that work synchronised, as suggested by Ruzicka and Marshall [1] Another requisite of SIA is the use of microprocessor control, also employed for data acquisition. Moreover, it is necessary to develop specific software to control the system, and many researchers have developed programs written in different programming environments [2–5].

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The large number of different applications described in the last decade demonstrates the versatility of SIA. Several food and beverage analyses using different detection techniques such as spectrophotometry [6,7], turbidimetry [8], voltammetry [9] and atomic spectroscopy [10] have been reported. Also, many application fields have been covered such as bioprocess monitoring [11], immunoassay [12], pharmaceutical [13,14] and environmental analyses [15–17].

Previous works have used SIA systems for automatic calibration and determination of single analytes, whereas there is a recent trend in the use of automatic systems for multiple analyte determinations employing high-dimensional signals, such as a part of a spectrum or a voltammogram [18].

These determinations sometimes exhibit heavy signal overlapping that impedes simple linear calibration fits. Useful chemometrics tools can be used for this purpose, such as multilinear regression, partial least square regression, and artificial neural networks (ANNs). The combination of SIA with chemometric tools has already been reported [19–22].

It is sometimes difficult to quantify or even detect a single analyte when it is in a complex matrix that interferes with the signal. Some analytical techniques permit good performance but they need expensive equipment and skilled personnel. In an attempt to obtain a simple, cheap and robust analytical system for several analytes in the presence of interferents, the use of overlapped signal and arrays of sensors have been proposed as an alternative. With these principles, the terms electronic tongue [23] for liquid samples or electronic noses [24] for gas samples have recently been coined. Some works, those on taste sensors, seek to mimic specifically the human sense of taste [25].

When high dimensionality of the data has to be treated, appropriate tools must be used. ANNs are able to extract the information required in presence of interference effects [26]. ANN algorithms act as “black-box” models, showing special abilities in describing non-linear responses obtained with different sensors. These algorithms use a learning capacity, extracted by the generalisation from a large amount of starting data. The great amount of data points (sometimes over one hundred) needed by the ANNs for their learning is one of the disadvantages encountered in this type of calibration. Apart from that, great care must be taken when choosing the training (or learning) data set [27] because of the poor capacity of the algorithm to extrapolate. Due to this fact, concentration space must be perfectly defined, including maximum and minimum concentrations of all the analytes in this learning data set.

This work presents the development and application of a SIA system to be used with higher dimensional data generated with electrochemical sensors. Only two previous close-related works are located in the literature, one dealing with the determination of metals with an array of ISEs in an FIA system [28], and other from our laboratory describing the determination of nitrate in the presence of chloride interferent [29]. Other works employ similar FIA systems to obtain electronic tongues, but departing from voltammetric sensors [30]. A related case is that of “artificial taste”, which can employ an impedance measurement [31], and is devoted to mimic the human sense response. In the presented work, an SIA system employing potentiometric sensors is firstly illustrated, followed by the biparametric amperometric calibration for metals. Finally, the SIA system is applied in the automated generation of the training data needed for a voltammetric electronic tongue, which is modelled employing ANNs. The final analysis system is aimed at quantifying oxidizable compounds, normally found in drug formulations.

2. Data analysis

ANNs act inspired in a human brain. The basic processing unit is called “neuron”, each one featuring local memory capacity. These neurons are structured in layers, with connections between all of them. Information is introduced into the algorithm through the input layer. This layer transmits the information to the hidden layer/s and the information is processed in parallel through the network, weighted by a transfer function, according to the importance of that specific data. There are various types of ANNs, but in electrochemical determinations, feedforward backpropagation neural networks is the normal choice [27,32]. Learning of this algorithm is based on finding how important is each aspect of the input information in order to obtain the expected outputs. For doing this, the network changes the importance of all connections (weights) between neurons. The building of the ANN model consists of finding the proper set of weights from the training information.

Two further issues should be considered in order to obtain an appropriate model: network topology and training strategy [32]. The network topology defines an optimum number of neurons, layers and the transfer functions used. It is common to use three-layered networks (input, hidden and output layers), where the input layer has the same number of neurons as the dimension of the input information. The number of neurons in the output layer is equal to the number of substances determined. The number of the hidden layer is optimised by trial and error [26]. Additional hidden layers can be added, but this is seldom used in chemical analysis applications.

The other consideration is the interactive training strategy, where accuracy and precision requirements are fulfilled if convergence is achieved. It is in this consideration where data points must be divided in at least two sets: training and test subsets. The training set is used to build the proper modelling of the response, being necessary a large amount of data for this purpose. The test set is used in order to check externally the prediction ability of the built model.

The network is ready to quantify when the training algorithm builds a model that has reached a prediction value below a preset amount. The more data points used in the training set, the better learning would be achieved. This could seem an advantage but it is sometimes, a drawback when networks are overfitted, and they are not capable of predicting accurately. An additional care must be taken regarding to the overfitting problem mentioned in ANN. A good way to avoid it, is to estimate the generalization error during the training process [32].

The need of a large and appropriate data set for the correct learning of the algorithm suggests the use of automatic systems in handling and preparing samples, thus facilitating the attainment of a properly trained electronic tongue. Some researchers have already used automated systems for this purpose [33,34].

3. Experimental

3.1. Reagents

All reagents employed were of analytical grade unless specified. Doubly distilled water was used throughout.

In the potentiometric determinations, carrier solution used was 0.01 M Imidazole (Fluka) buffer with its pH adjusted to 6.5 with HCl. Potassium, sodium, ammonium, and calcium ion solutions were prepared dissolving the right amount of its chloride salts (Fluka) in carrier solution.

For amperometric determinations of heavy metal ions, stocks solutions were prepared by appropriate dilutions from 1000 mg l^{-1} stocks (Fluka standards for atomic spectroscopy) in a media at pH 3 (HCl acid) and 0.1 M KCl (Merck), in order to assure high conductivity. Determinations of ascorbic acid, 4-aminophenol and 4-acetamidophenol (paracetamol) in the 0.01–0.5 mM range were developed from solutions 10^{-3} M prepared by dissolving pure substances (Fluka) in KCl 0.1 M.

3.2. The SIA system

The designed SIA system is shown in Fig. 1. The liquid pumping system consists in a bi-directional microburette (Precision Syringe drive/2 Module, Hamilton, Switzerland), using syringes of 1, 2.5, and 5 ml (Hamilton), depending on the application. The selection valve was a motorised MVP valve with a six-way valve head, HVXM 6-5 (Hamilton).

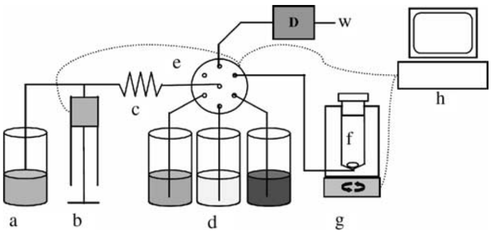

Fig. 1. Manifold of the developed SIA system: (a) diluting solution, (b) microburette, (c) holding coil, (d) stock solutions, (e) selection valve, (f) mixing cell, (g) magnetic stirrer, (h) personal computer. D, detector; W, waste.

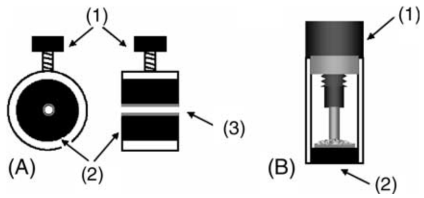

Fig. 2. (A) Scheme of the tubular flow-trough ISE design and its PVC membrane; (B) scheme of the amperometric transducers made from the epoxy graphite composite. (1) electrical connector, (2) epoxy-graphite composite, (3) PVC ion selective membrane.

SIA components were connected together with PTFE tubing (Bioblock, France) with 1 mm i.d. employing fittings for low-pressure chromatography. The holding coil was also made from this tube and the inner volume was set to 10 ml, in order to minimise contamination of syringe drive caused by samples or stock solutions.

A magnetically stirred mixing cell with computer control was developed to assure homogeneity in sample preparation; it consisted in a 10 ml cavity in a Perspex block. The cell was designed with a single input–output way, in a conical bottom for complete voiding.

Microburette selection valve as well as the mixing cell stirrer were entirely controlled by a PC computer with in-house software programmed in BASIC (Quick-Basic, Microsoft, USA). Variation of commands in an independent level text file enables different sequences of liquid handling by SIA system, being versatility one of the advantages of this concept.

First characterisation of the SIA system was carried out using absorbance reading of a phenol red solution. This analyte was selected because of its colour, which permitted the easy following of liquids and allowed the precise timing for every step in sample holding and preparation. Once characterised, the SIA system was used in analytical determinations.

A sample cycle was performed in typically 5 min, including aspiration of solutions, homogenisation, pumping into detection system, and cleaning prior to the next cycle.

3.3. Detectors

Different electrochemical techniques have been implemented, each of them with home-made electrodes or transducers, constructed with existing technology in our laboratories.

3.3.1. Potentiometry

Potentiometric measurements were developed with tubular flow-through ion-selective electrodes (ISEs), consisting in a perspex cylinder body, filled with an internal solid contact made from an epoxy-graphite composite, as normal detector in our laboratories [35]. The selective membrane is deposited, covering the inner wall of a hole drilled axially the inner solid contact, as shown in Fig. 2(A). Membrane formulations were standard ammonium ISEs employing nonactin and bis(1-butylpentyl)adipate (both from Fluka) [36], and ionophoric antibiotic for sodium employing monensin and dibutylsebacate (from Acros and Fluka, respectively) [37].

Computer-controlled detection was achieved employing a digital potentiometer (Crison 2002, Crison, Spain), and its serial communications line with home-made data-acquisition software. Reference electrode was a double-junction electrode (90-02-00 model, ThermoOrion, USA) inserted in the flow system. As all potentiometric measurements were performed with identical ionic media, activity coefficients were supposed constant.

3.3.2. Amperometry

Amperometric measurements were carried out with electrochemical transducers made from an epoxy-graphite composite constructed as described previously [38,39]. Epoxy resin used was Epotek H77 (Epoxy Technology, USA). Measurements were performed with an Autolab/PGSTAT20 electrochemical system (Ecochemie, Netherlands).

The amperometric transducers, schematised on Fig. 2(B), were inserted in the flow system with a specially designed flow cell having a low dead volume, and a stainless-steel base acting as the auxiliary electrode. The reference electrode used here was the same as in potentiometric experiments.

3.4. Procedures

3.4.1. Potentiometric calibrations

The flow system is programmed to prepare and deliver a specified multianalyte standard, by mixing appropriate volumes of the different stock solutions and retaining them in the holding coil. After that all volumes are pumped into the mixing cell for homogenisation. Once this is done, the prepared solution is re-aspired and pumped to the potentiometric sensors where the readings are performed by the PC.

3.4.2. Amperometric calibrations

In simultaneous measurements of lead and cadmium, a similar procedure to the latter was taken. The preparation of calibration standards done by the SIA system permitted calibration prior to sample measurement. In this case to improve detection limits, an anodic stripping voltammetry (ASV) technique with differential pulse (DPV) was selected. Experimental conditions employed in this determination are: 180 s potentiostatic deposition time at −1.2 V, scanned voltage from −1.0 to −0.3 V, modulation amplitude 20 mV, pulse time 70 ms, and scan rate 10 mV/s. Metal concentrations ranged from 1 to 4 μM (0.2 to 0.8 mg l^{-1}) for lead and 15 to 30 μM (1.7 to 3.4 mg l^{-1}) for cadmium.

In the voltammetric e-tongue application, simultaneous determination of oxidizable compounds – ascorbic acid, 4-acetamidophenol and 4-aminophenol – was developed by use of the SIA system. Determinations were performed in this case through the linear sweep voltammetry technique. Potential was scanned from 0 to 1.5 V at 0.1 V/s, with a potential step of 10 mV. Intensity readings were directly taken at each applied potential.

Given that ANNs were going to be used to build the calibration model, a random set of standards was generated. This set consisted of 65 experimental solutions with a different concentration value for each of the considered species, which were prepared individually by the SIA system. Custom software was prepared to determine, from a random numbers algorithm, the concentration of each substance in each standard. With this provision, we can assure the complete independence between samples, a desirable condition for ANN modelling.

3.5. Software

Neural Network processing was developed with Matlab 6.0 (Math works), using its Neural network toolbox (v. 3.0). Sigma Plot 2000 (Jandel Scientific, Germany) was used in pre-treatment, non-linear fittings and in graphic representation of the data.

4. Results and discussion

4.1. Characterisation of potentiometric sensors

Among the most important parameters for characterisation of ISEs, their selectivity coefficients (k^{pot}) towards interfering ions receive special attention. The recommended determination is that of the Mixed Solution Method, [40] obtained by manual calibration. The automatic preparation and handling of samples by the SIA system facilitates a fast determination of these values.

Some selectivity coefficients were determined using the SIA system calibration for NH_4^+ and Na^+ ISEs. Their determination consisted of successive dilution of concentrated solutions of the primary ion, while maintaining constant the interference concentration. The sequence of dilution can be summarised by: aspiration of the right amount of concentrated solutions, aspiration of the right amount of dilution solution, impulsion of all the solutions towards the mixing cell, stirring, aspiration of the prepared solution, impulsion towards detector, and detection. Serial dilutions can also be performed if necessary.

The set of solutions describes the complete behaviour of the electrode, which enables the value for the selectivity coefficient ($k_{x,y}^{\mathrm{pot}}$) in the Nikolskii–Eisenmann equation to be established:

$$\Delta E = A + B \log(C_x + k_{x,y}^{\mathrm{pot}} C_y^{Z_x/Z_y}) \quad (1)$$

where Z_x and Z_y are the charges of the primary and interfering ions at concentrations C_x and C_y, respectively.

Two different experiments were carried out to obtain values of selectivity coefficients. The simplest was a single calibration with a fixed concentration of interference concentration, as is normally performed in potentiometry. The typical curvature at a low concentration of the primary ion

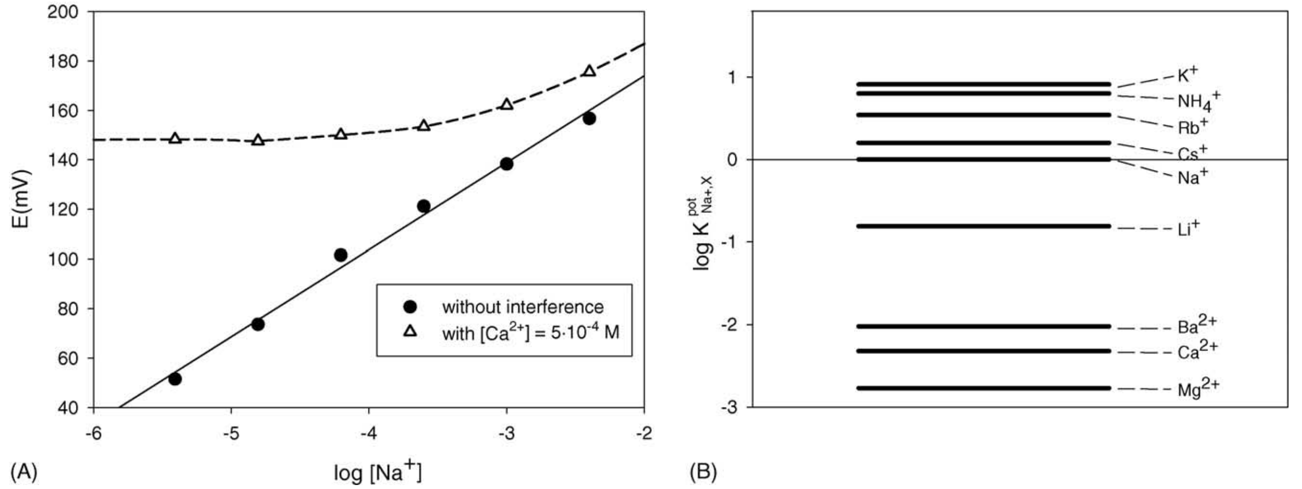

Fig. 3. (A) Automated calibrations with and without interference. Interference is studied by the Mixed Solution Method at a fixed activity of the interfering ion. (B) Selectivity coefficients for different interference ions using a sodium ISE.

was observed, and the experimental fitting of data points to the Nikolskii–Eisenmann equation allowed the k^{pot} value to be obtained.

Fig. 3 shows the automated characterisation of PVC-membrane sodium ISE in use in our laboratories. With the aid of the developed SIA system, a full interference characterization for such a new sensor can be easily completed with minimum effort.

Higher dimensionality of data permits obtaining more reliable values of selectivity coefficients, as long as the fitting models the electrode behaviour in the presence of different interference and analyte concentrations. For this purpose, several calibrations with varying interferent and primary concentrations were carried out. The fitting of the whole data set exhibits the new k^{pot} values with some differences between single calibration and multiple calibration adjustments. Fig. 4 shows the two-ion calibration of an ammonium ISE in the presence of potassium as interferent together with the fitted Nikolskii–Eisenmann response surface. There an earlier curvature of calibrations can be observed as the potassium concentration increases; the typical behaviour expected for an interference effect. The $\log k^{\mathrm{pot}}_{\mathrm{NH}_4^+,\mathrm{K}^+}$ obtained from the non-linear surface fitting was -1.26, in agreement with results obtained by classical means [36,41], which is -1.0.

4.2. Calibration using DPV signals

The first measurements developed in amperometry consisted of simultaneous calibrations of lead and cadmium in water. Calibrations equivalent to those performed in potentiometry were carried out. The transducers used for the stripping of metals were epoxy-graphite electrodes, as shown in Fig. 2(B). Voltammograms with two peaks were obtained, one appearing at the re-oxidation potential of cadmium for this transducer (ca. -0.8 V), and the other consisted of the re-oxidation of lead (ca. -0.5 V).

Dual calibration was obtained by automatically preparing different solutions with varying concentration of both analytes. Fig. 5(A) shows some of these voltammograms; as the data set consisted of four variables (concentration of lead, concentration of cadmium, scanning potential and measured currents), a transformation was made to enable visualization. For this purpose, voltammograms are displayed in a sequence of increasing concentration of metals. As no overlapping of the signal is present in this case, the simple reading of peak current allowed the separate calibration for each metal as is shown in Fig. 5(B). Using the statistics of the fitted regression line [42], the detection limits for each metal were calculated. These values were 3.1 μM for cadmium determination and 0.4 μM for lead.

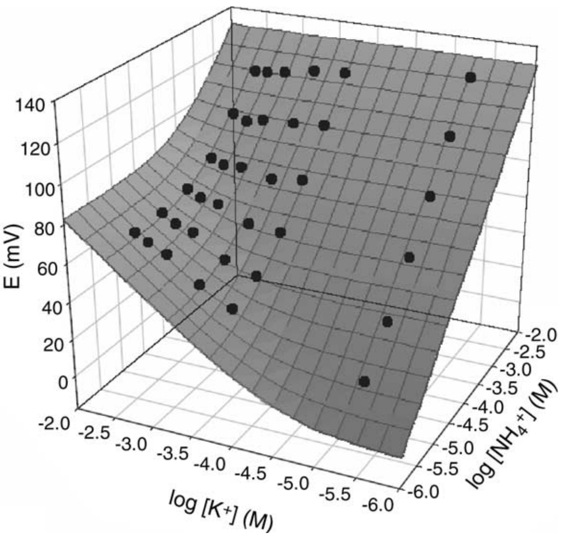

Fig. 4. Surface response of an NH_4^+ ISE in presence of K^+ varying both concentrations. Fitted model: $E = A + B \cdot \log([NH_4^+] + C \cdot [K^+])$.

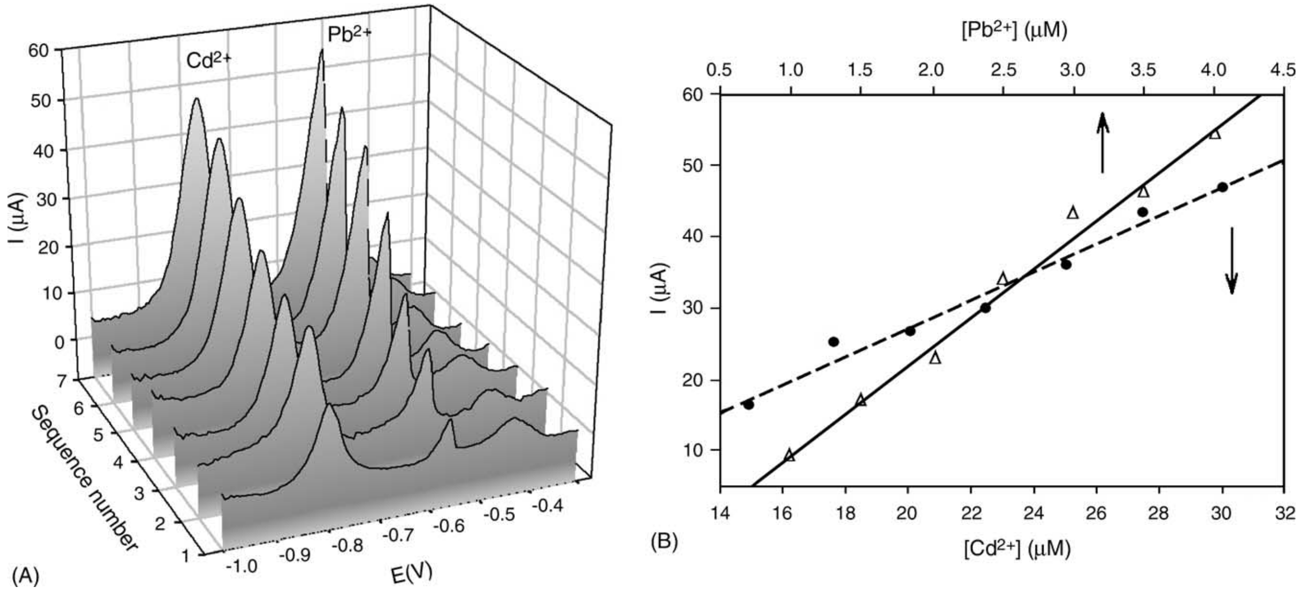

Fig. 5. Dual calibration of lead and cadmium employing the SIA system. Automated sequence of voltammograms and separate calibration for the determination of lead (△) and cadmium (●).

4.3. Voltammetric electronic tongue

After calibration of separate analytes, a group of oxidizable substances – presenting similar oxidation potentials and so with some degree of overlapping – was tested with the described voltammetric detection system.

Ascorbic acid, 4-acetamidophenol and 4-aminophenol was the three-component case studied. Sixty-five sample solutions, with their concentrations defined randomly, were prepared by appropriate dilution schemes performed employing the SIA system, which also allowed the measurement of the corresponding voltammetric signals. The concentration of ascorbic acid was between 12 and 410 μM, the 4-aminophenol concentration between 17 and 530 μM and the 4-acetamidophenol concentration between 10 and 420 μM – the three ranges selected in preliminary linearity tests. Fig. 6 shows the 3D plot of the randomly generated standards. The subsequent modelling was performed employing ANNs.

Fig. 7 shows a sample of different voltammograms obtained using different standard concentrations. As we can observe, strong overlapping is present, a circumstance that points to non-linear calibrations with ANNs. The appearance of ascorbic acid and 4-aminophenol signals is around +0.3 V, while paracetamol appears at +0.6 V. In figure, voltammograms (A) and (C) present a similar response which is around +0.3 V due to the similarity in the ascorbic acid and 4-aminophenol concentration, but a different signal at +0.6 V because of the higher concentration of paracetamol in voltammogram (A). Voltammogram (B) presents a high signal at a voltage of +0.3 V because of the high concentration of both ascorbic acid and 4-aminophenol.

The initial data set was formed by a group of over 60 voltammograms with 135 intensities, each associated with 135 potentials. As the departure information was too complex, a discrimination of three out of four data points was done. Besides, the resulting reduced data set was subdivided randomly in three subsets in order to obtain the ANN model: training, validation and external test sets, having 50, 25, and 25% of the original data, respectively.

After obtaining and determining the data set from our experiments, backpropagation ANNs with different topologies were tried, in which we varied the learning algorithm as well as the number of neurons in the single hidden layer used. All the ANNs built had 35 input neurons – the reduced voltammogram and three output neurons – the concentrations of the three analytes. Preliminary tests discouraged us from using

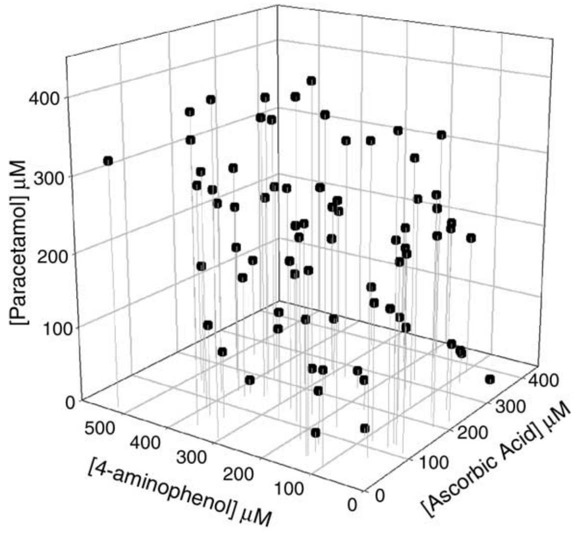

Fig. 6. Randomly generated concentration space (65 points) for simultaneous calibration of ascorbic acid, 4-aminophenol and paracetamol.

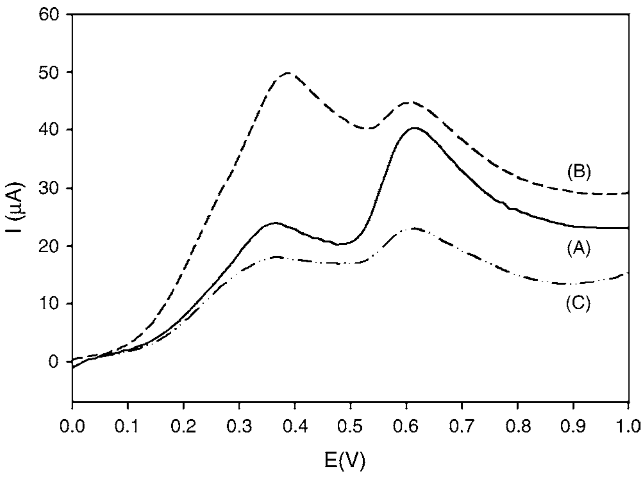

Fig. 7. Voltammograms obtained for three mixtures containing different amounts of the three oxidizable compounds. The high degree of overlapping is illustrated. Concentrations used (ascorbic acid, 4-aminophenol and paracetamol, respectively), are (A), 143, 481,and 270 μM; (B) 214, 525, and 242 μM; (C) 114, 516, and 178 μM.

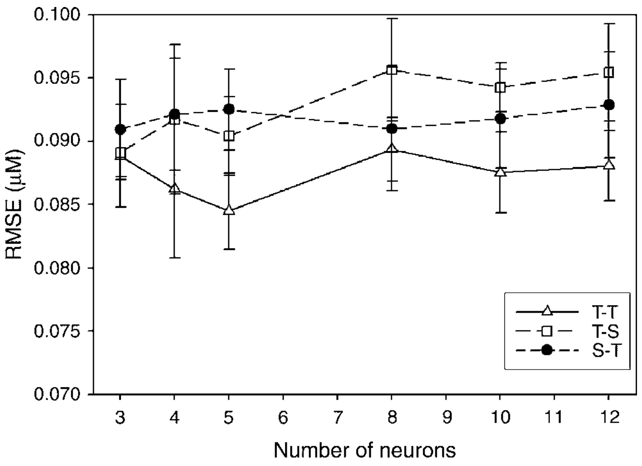

Fig. 8. RMSE values for the test set obtained for networks employing a different number of neurons on its hidden layer, and the three combinations of transfer functions studied in detail. (T: Tansig transfer function, S: Satlins transfer function).

the gradient descent-learning algorithm because of the lack of convergence noticed for the selected conditions. The first successful approach consisted of a feedforward backpropagation ANN with the Levenberg–Marquardt “early stopping” algorithm using different combinations of transfer functions, as well as different topologies, i.e. with varying number of nodes in the hidden layer.

Feedforward backpropagation employing the Bayesian regularization training algorithm was also tried, as long as the Levenberg–Marquardt algorithm showed overfitting problems, with correct training but bad prediction capacity though Bayesian regularization applies statistical methods to detect neurons causing overfitting. With this probabilistic algorithm better models were achieved [43]. Moreover, when using this algorithm, only two subsets of data are needed, training and external test (with ca. 2/3 and 1/3 of the data, respectively).

With Bayesian Regularization algorithm selected, an exhaustive search for the best neural network configuration was performed. Three transfer functions for the hidden and output layers were initially selected. Thus, combinations of Tan-Sigmoidal (Tansig), Sat-Lineal (Satlins), and Pure lineal (Purelin) transfer functions [44] were tested. First, a quick study was done with seven different combinations of the above three functions in the hidden and output layers, employing ANN structures having 4, 8, and 10 neurons in the hidden layer. The configurations with minimum prediction errors or lower RMSE values, obtained after training for 5000 epochs, permitted us to select the pairs Tansig-Satlins, Tansig-Tansig, and Satlins-Tansig as better combinations. The General features observed were reduced prediction errors, with total RMSE (root mean square error) values below 0.15 μM in all cases, and a worst behaviour for the purelin–purelin pair.

For the final optimisation of the ANN configuration, an exhaustive scan of topology combinations were developed for the three chosen pairs of transfer functions. For these combinations, five replicates of the modelling for each architecture (3, 4, 5, 6, 7, 8, 9, and 10 nodes) were performed, in order to find not only the configuration with minimum prediction errors, RMSEs, but also that with the best prediction ability.

Fig. 8 shows the RMSEs values, mean and dispersion of the five replicates for the three best combinations. The study concluded by showing that three neurons is the configuration that yields minimum residual errors for Tansig-Satlins and Satlin-Tansig combinations as well as lower confidence intervals. A minimum with the Tansig-Tansig combination is observed when five neurons are used in the hidden layer, but the general trend, the larger confidence interval, and the subsequent study of prediction behaviour suggested us to reject this architecture.

The final choice of the ANN architecture was done by observing the comparison graphs for each determined substance in the external test set – the data not participating in training. These comparison graphs were constructed plotting expected and calculated values, and were compared with the ideal comparison line ($y = x$), with slope 1 and zero intercept. Representative trainings with the three combinations are summarised in Table 1, together with their correlation coefficients. Observing this global data, the Tansig–Satlins transfer function pair with the architecture of 35 × 3 × 3 neurons was chosen as the best ANN.

This combination showed the best correlation coefficients for two out of three analytes as well as better slopes and close to zero-intercepts in many cases. A compromise between the minimum RMSEs values, and the best slopes and correlation coefficients of the external test had to be assumed.

Adjustment of the data by this ANN exhibits good prediction ability for the test set, with data not participating in the learning process. The degree of fitness is visualised for one of the training replicates by plotting the comparison graphs for the three considered substances (Fig. 9). The proximity to

Table 1
Comparison parameters of the modelling ability of the three best network configurations calculated using the external test subset (obtained /vs./expected, $y = \mathrm{m} \cdot x + \mathrm{b}$)

	m			b			r		
Combination	T-T	T-S	S-T	T-T	T-S	S-T	T-T	T-S	S-T
Ascorbic acid	1.014 ± 0.031	0.986 ± 0.024	1.006 ± 0.043	$-4.1 \times 10^{-3} \pm 5 \times 10^{-3}$	$-3.6 \times 10^{-2} \pm 4.0 \times 10^{-3}$	$-3.9 \times 10^{-2} \pm 7.5 \times 10^{-3}$	0.876 ± 0.017	0.898 ± 0.005	0.875 ± 0.024
4-Aminophenol	0.953 ± 0.055	0.955 ± 0.060	0.865 ± 0.018	$-2.7 \times 10^{-3} \pm 1.2 \times 10^{-2}$	$-1.9 \times 10^{-2} \pm 1.2 \times 10^{-2}$	$7.4 \times 10^{-3} \pm 7.7 \times 10^{-3}$	0.941 ± 0.007	0.939 ± 0.005	0.934 ± 0.006
Paracetamol	1.050 ± 0.039	1.043 ± 0.029	1.034 ± 0.017	$-2.9 \times 10^{-2} \pm 6.4 \times 10^{-3}$	$-3.4 \times 10^{-2} \pm 2.9 \times 10^{-3}$	$-2.3 \times 10^{-2} \pm 3.7 \times 10^{-3}$	0.954 ± 0.003	0.977 ± 0.005	0.949 ± 0.006

Conditions correspond to three neurons in the hidden layer and the three best combinations of transfer functions in the hidden/output layers. (T-T, Tansig-Tansig; T-S, Tansig-Satlins; S-T, Satlins-Tansig). Uncertainties are calculated at the 95% confidence level.

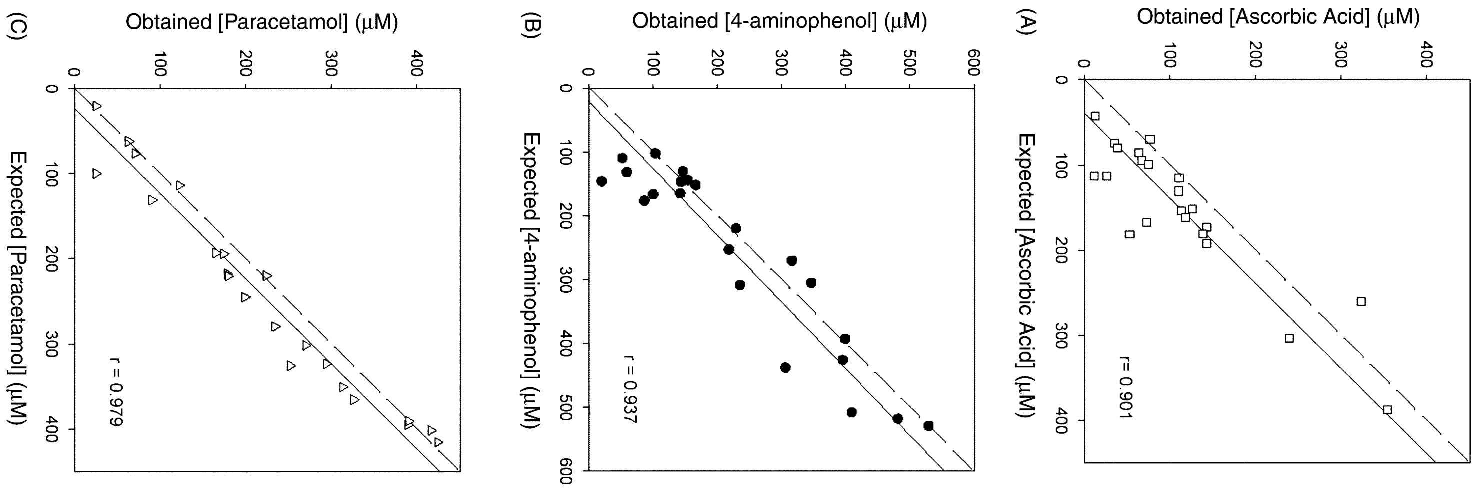

Fig. 9. Correlation between expected and obtained concentration values for the three oxidizable substances considered: (A) ascorbic acid; (B) 4-aminophenol; (C) Paracetamol. The solid line is the fitted regression line (correlation coefficients are displayed on each graph) and the dotted line the theoretical comparison $y = x$ line.

the theoretical comparison lines validates its use for the determination of this family of compounds. From the figures, it can be observed that best results are obtained for paracetamol than for ascorbic acid or 4-aminophenol, given these two were easily oxidized in air.

Correlation coefficients (r) showed a very good significance. A t-test was performed in order to prove that significance [40]. From the data points of the test set, a t value was calculated by the equation:

$$t = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}} \tag{2}$$

Calculated statistics for n-2 degrees of freedom were 9.29, 11.99, and 21.47 for ascorbic acid, 4-aminophenol and paracetamol respectively. In all cases the calculated values are greater than the tabulated value, (at the 99% of level) which is 2.85, so the significance of the correlation achieved with each substance is demonstrated.

5. Conclusions

A SIA system was developed in order to manipulate and prepare samples and standards for automatic calibrations aimed at higher dimensional signal applications. Different detection methods were used so that the versatility of the system was shown: selectivity coefficients estimation with two degrees of freedom was illustrated, and a satisfactory dual calibration for heavy metals using a full voltammogram is demonstrated. A complete electronic tongue modelling for oxidizable compounds present in drug formulations was achieved with automated generation of information (standard solutions) for learning. Results show that the SIA system is capable of resolving different higher order analytical situations suited to electrochemical detection, just by fitting different detection systems and changing some components of the manifold. Further work using ISE arrays or voltammetric detection is still carried out in our laboratories. The main goal is to obtaining electronic tongues for real samples in presence of interferences, using more selective detectors such as enzymatic biosensors or catalysers.

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