

*Original Paper*

**Automatic sequential injection analysis electronic tongue with integrated reference electrode for the determination of ascorbic acid, uric acid and paracetamol**

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Received March 1, 2006; accepted June 26, 2006; published online September 29, 2006

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**Abstract.** A new approach towards a voltammetric electronic tongue has been developed. Automation of the system was achieved by the use of a sequential injection analysis (SIA) system. Design and construction of a small detection device containing 3 working electrodes was carried out. Platinum, gold and epoxy-graphite discs were used for this purpose. An Ag/AgCl reference electrode was integrated into the measuring cell in order to minimise electrical noise. Three oxidisable compounds of clinical interest i.e., ascorbic acid, uric acid and paracetamol, could be quantified by the system. Employing the voltammograms as departure information, artificial neural networks (ANN) have been used as chemometric tool for the modelling of the system.

**Key words:** Electronic tongue; SIA; neural networks; ascorbic acid; uric acid; paracetamol.

Analytical determinations in samples containing different compounds is sometimes difficult due to interfering overlapping effects. The use of separation stages such as in chromatography, can sometimes avoid this drawback but it is normally an expensive option. Alternatives presented some years ago are the multivariate analytical systems, already established i.e., with spectrophotometrical determinations. Nowadays, there

are multivariate systems based on the use of chemical sensors, as for example electronic tongues [1, 2]. These systems pretend to mimic human taste sense [3]. For this purpose an array of non-specific sensors is used as detection system, trying to obtain cross-sensitivity in their measurements.

Several electronic tongues have been developed in the last years, especially in qualitative analysis in industrial process control [4], environmental monitoring [5–8] or food industry [9–12]. Quantitative applications are much less developed and literature in this trend is not as significant as that for the qualitative determinations, but it is of enough importance to be considered [1]. Both potentiometric and voltammetric tongues have been developed as found in the bibliography [5, 13]. In the case of voltammetric electronic tongues there are some important works that can be found in the literature, specially those concerning the Swedish group from Linköping [4, 5, 10–12, 14, 15].

One of the features that sometimes are considered as a drawback when using electronic tongues is the huge amount of previous knowledge or data needed for the modelling or training of these systems. Because of this fact, automation in sample or standard solutions handling and preparation is an alternative in order to reduce time and reagent consumptions. Combination of automatic flow systems and electronic tongues has been already reported [14–17].

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In this communication we attempt the determination of mixtures of oxidizable analytes of pharmaceutical interest. Namely, the determined species are ascorbic acid, uric acid and acetaminophen (paracetamol). For their determination, a voltammetric electronic tongue is proposed coupled with artificial neural networks (ANN) as chemometric tool for modelling the analytical system. A sequential injection analysis (SIA) system has been used for the automated operation of the electronic tongue, so allowing for the automatic generation of the information needed for modelling the response.

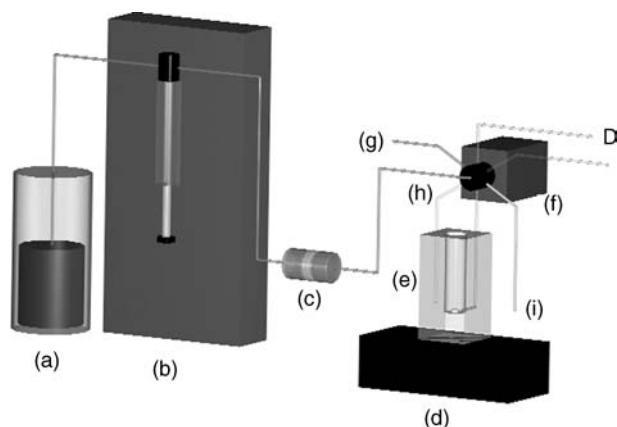
## Experimental

### Reagents

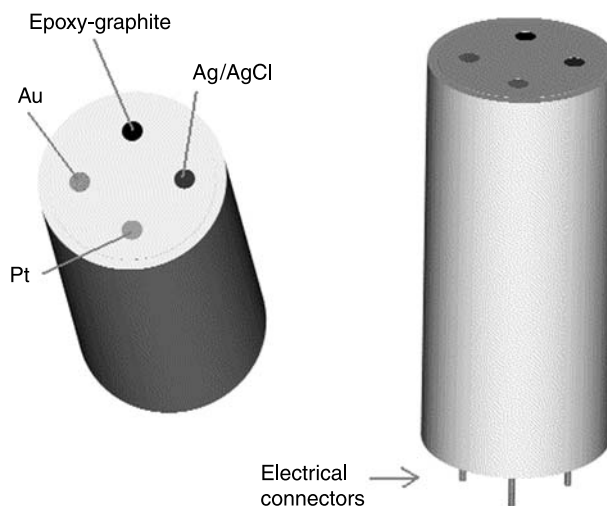
All reagents employed were analytical grade unless specified. Doubly distilled water was used throughout. Stock solutions needed for all experiments were prepared by dissolving the appropriate amount of pure substance in 0.05 M KCl solution. KCl was used for ensuring high conductivity media, and to fix chloride ion concentration for the Ag/AgCl reference half-cell.

### The SIA system

The designed SIA system is shown in Fig. 1. As pumping device a bi-directional microburette (Precision Syringe drive/2 Module, Hamilton, Switzerland) equipped with a 2.5 mL syringe (Hamilton) was used. Selection valve was a motorised MVP valve with a 6 way valve head, HVXM 6-5 (Hamilton). Previous works showed the high efficiency of the developed system [17]. PTFE tubing (Bio-block, France) with 1 mm i.d. tube with fittings for low-pressure chromatography was used as connecting manifold. A 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 Perspex block with a 10 mL conic



**Fig. 1.** sequential injection analysis system design. (a) Diluting solution, (b) bi-directional microburette, (c) holding coil, (d) magnetic stirrer, (e) mixing cell, (f) selection valve (with 1 input and 6 outputs), (g) ascorbic acid standard solution, (h) uric acid standard solution, (i) paracetamol standard solution, (D) detector



**Fig. 2.** Design of the working electrode array. Au, Pt and epoxy-graphite disc act as working electrodes. Ag previously oxidised into a Ag/AgCl disc acts as reference electrode

cavity was used as mixing cell. Automatic control of stirring was used for ensuring complete homogeneity of the prepared solutions. Microburette, selection valve, as well as the mixing cell stirrer were entirely controlled by a PC computer with 3-level-communication in-house software, programmed in BASIC (Quick-Basic, Microsoft, USA). Simple 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.

### Electrode array

A new approach to the electronic tongue is achieved by the use of a novel small array of working electrodes with inner reference electrode. Figure 2 shows the design of the quatrielectrode used. Platinum, gold and epoxy-graphite 1 mm diameter discs were used as working electrodes. Ag electrode was previously oxidised in presence of chloride ion in order to obtaining an Ag/AgCl reference electrode (chloride ion needed is present in the carrier solution). All four electrodes were fabricated from its metal wires and encased in epoxy resin (Epotek H77, Epoxy Technologies) in a 6 mm inner diameter PVC tube used as body.

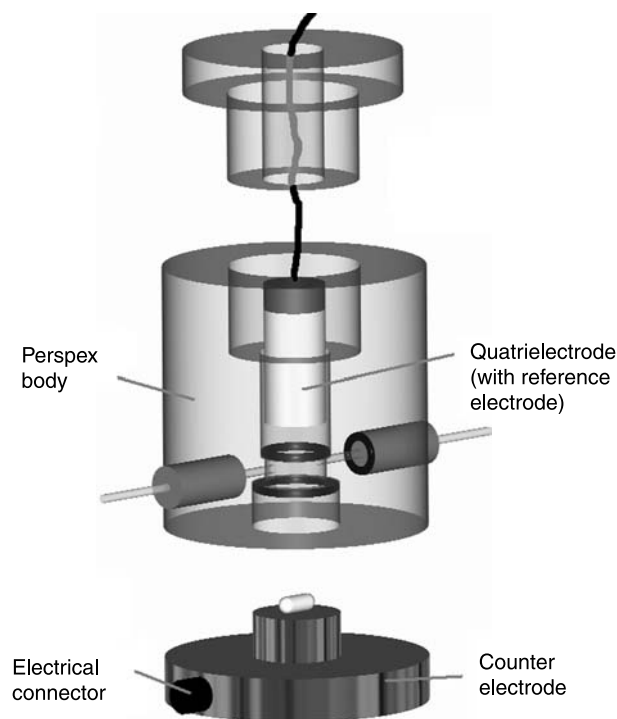
### Detection flow cell

Detection flow-through cell design is shown in Fig. 3. Previous work [18] used the same measuring cell with excellent results. A principal advantage of this measuring cell is the use of stainless steel as base material and so, as counter electrode. By doing this, working, reference and counter electrodes are integrated inside the small volume (140  $\mu$ l) of the measuring cell, allowing operation with reduced electrical noise.

### Procedures

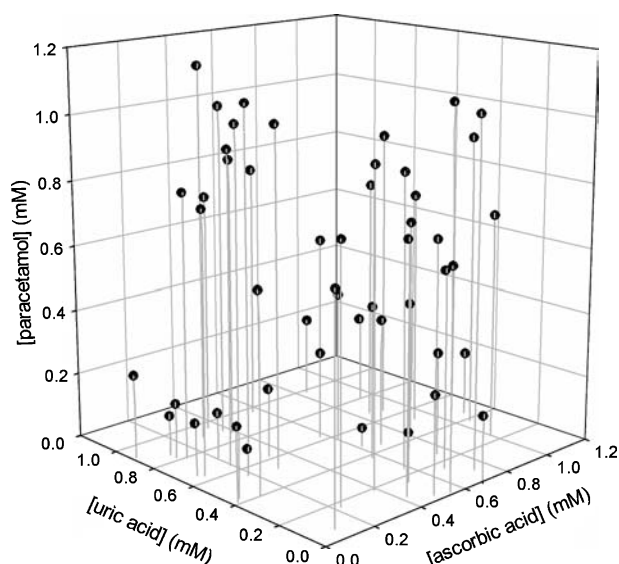
#### Automated preparation of standards

A total amount of 50 standard solutions were prepared automatically by the SIA system, by dilution of the appropriate amounts of the stock solutions. Automation is achieved by the high versatility of programming different specific files out of the control program. The



**Fig. 3.** Measuring cell design. Stainless steel base acts as counter electrode. Quatrielectrode with integrated reference electrode is also shown

sequence of preparation of each standard and measuring cycle can be summarized as follows; system cleaning with dilution solution, aspiration and retention in the holding coil of specific volumes of the analytes, pumping of the total volume into the mixing cell, appropriate addition of diluting solution to a final volume of 2 ml, stirring and homogenisation, aspiration of the prepared solution into the



**Fig. 4.** Three-dimensional plot of the prepared dilutions. Automatic random concentrations are achieved, so independence between dissolutions is obtained

holding coil, pumping into detection cell and, finally, voltammetric detection. Cleaning steps are performed before a new solution is prepared. Figure 4 shows random distribution of the 50 prepared standards in a 3D view. Random distribution was preferred in order to avoid concentration coincidences in, at least one component that experimental design normally offers in a factorial distribution of experimental points.

#### Detection procedures

Linear sweep voltammetry was used as detection technique. Scans from 0 to 0.8 V were performed, while stirring at a prefixed speed, for each solution at a scan rate of 0.1 V/s with a potential step of 8 mV, so a total of 101 currents were recorded. Measurements were performed using an Autolab/PGSTAT20 multichannel electrochemical system (Ecochemie, Netherlands). A total of 150 voltammograms were obtained, 50 for each working electrode. A whole sample preparation is performed in 5 minutes, including standard solution aspiration, homogenisation in the mixing cell, pumping into detector, detection and system cleaning. As long as obtained oxidation products did not show any fouling at the electrode surfaces, a simple hydrodynamic cleaning of the cell was enough to ensure measurements' stability. Electrode polishing or electrochemical cleaning was not necessary in the whole experiment's duration.

#### Data treatment and ANN modelling

##### Data pre-treatment

In order to find how significant each electrode was, ANN modelling for each electrode was firstly performed. It was observed that the three electrodes had meaningful information to be used. Addition of the three voltammograms obtained for each measured dissolution was performed in order to obtain a single data vector and thus reduction of the data matrix with good results [19]. By doing this a final  $50 \times 101$  matrix was obtained as input data for the ANN modelling.

##### ANN modelling

From the complete set, 35 of the prepared solutions were used in ANN training model and the remaining 15 were randomly selected as external test set, for comparison of performance. Two clear outliers in the training set were detected and so eliminated from the set, thus, 33 were the points available for training. Recorded currents were entered into the network as the input values and concentrations were established as targets. Figure 5 shows the schematic way of action of the proposed ANN modelling.

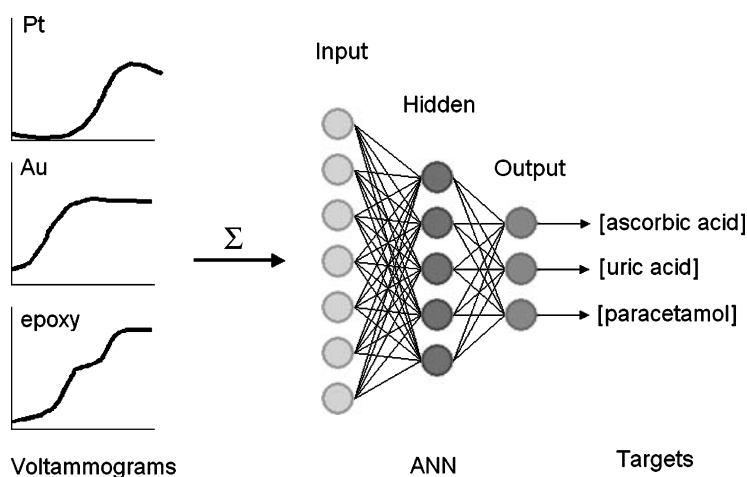
Learning estimation of the neural network was considered as the minimal error obtained between the established values and the found values of the target concentrations. Root mean square error was calculated during learning process according to:

$$\text{RMSE} = \sqrt{\frac{\sum_{ij} (c_{ij} - \hat{c}_{ij})^2}{3 \cdot n - 1}}$$

for each of the  $n$  samples ( $i$ ) and for each of the three analytes ( $j$ ) considered.

##### Software

ANN modelling using Matlab 6.1 (Math Works, Matick, MA) was employed, using its Neural Network Toolbox (version 4.0). Sigma



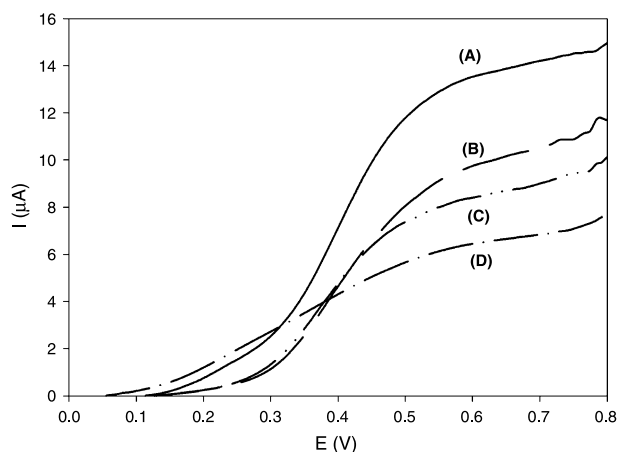
**Fig. 5.** Schematic ANN modelling approach. Addition of voltammograms is entered to the ANN in order to obtaining concentrations as target values

Plot 2000 (Jandel Scientific, Germany) was used in pre-treatment, linear fittings and graphic representation of the data.

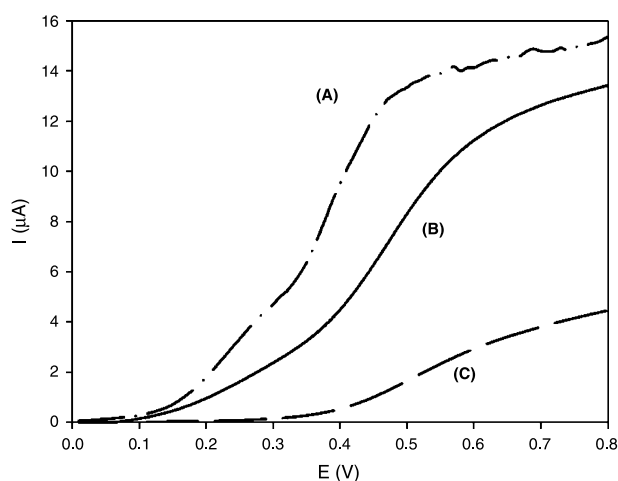
## Results and discussion

Figure 6 shows the different response presented at the Au electrode when measuring different solutions with variable concentration of the three analytes. As can be observed, very different overlapped signals are obtained depending on the amount of each analyte.

One of the desired features for the operation employing electronic tongues is the availability of differentiated response from each sensor used. That is, cross-sensitivity and absence of co linearity are desired behaviours for the sensor array used in these analytical systems. Figure 7 shows the obtained response of the three used electrodes for a given mixture



**Fig. 6.** Obtained linear sweep voltammograms at the Au electrode for different dissolutions (ascorbic acid, uric acid and paracetamol) (mM): (A) 0.841, 0.982, 0.249, (B) 0.049, 0.439, 1.066, (C) 0.016, 0.915, 0.258, (D) 0.679, 0.0, 0.206

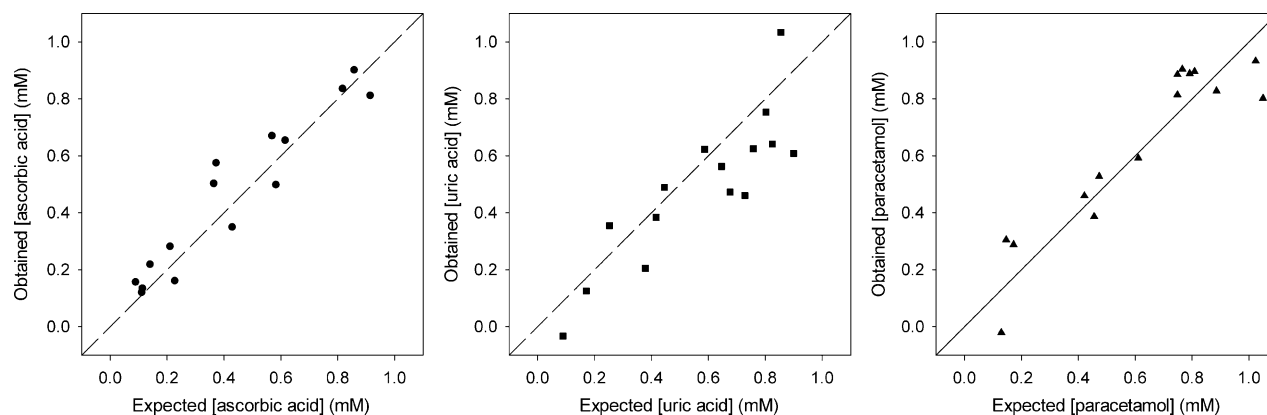


**Fig. 7.** Obtained linear sweep voltammograms for the three working electrodes: (A) Pt, (B) Au, (C) epoxy-graphite. Concentrations (ascorbic acid, uric acid and paracetamol) (mM): 0.930, 0.535, 0.722

of the three analytes. Each solution permits the obtaining of differentiated voltammograms, as well as different response for each electrode for the same solution, as can be observed.

For the obtaining of the response model with ANN, different network architectures as well as different learning algorithms were tested, such as gradient descent algorithm, Levenberg-Marquardt “early-stopping” and Bayesian Regularization. The latter was the final choice, as long as gradient descent and Levenberg-Marquardt algorithms showed heavy overfitting problems.

Once the learning algorithm was established, different architectures of the ANN were tested in order to obtain the optimal model. Input and output layers were established as the number of recorded currents and the



**Fig. 8.** Prediction capacity (test) of the developed voltammetric electronic tongue, for the three considered substances

number of analytes to be determined respectively. Study of the influence of the number of neurons in the single hidden layer was performed. Different transfer functions (sat-lineal, log-sigmoidal, tan-sigmoidal and pure-lineal) were also tested in the hidden and output layer in order to obtaining the best architecture.

Final configuration of the ANN model was  $101 \times 3 \times 3$  neurons with log-sigmoidal and pure-lineal transfer functions in the hidden and output layer respectively, extracted from minimum RMSE errors and correct modelling ability.

Figure 8 shows the comparison of the expected vs. the obtained values in the training model for three analytes with the optimal ANN configuration where a theoretical 1 slope and 0 intercept is nearly achieved. Besides, the prediction ability of the model is also shown in the figure for the three analytes but in this case for the external test subset. It must be remarked that these data is not employed at all at the modelling step, so goodness of fit is a measure of the accomplished modelling performance. In both cases, very good correlation was obtained with comparison lines (at the 95% confidence level), perfectly comparable with the theoretical behaviour. Prediction capability of the model could then be considered as very good.

Table 1 shows the regression parameters of the comparison lines for both training and test cases. It can be observed that less accurate results are obtained for uric acid. Ascorbic acid and paracetamol showed very good modelling and prediction capacity. Average observed errors were 8, 10 and 12% for the analytes ascorbic acid, uric acid and paracetamol, respectively.

**Table 1.** ANN model adjustments for training and test sets of the obtained data (uncertainty intervals at the 95% confidence level)

Analyte	Slope	Intercept	$r$
Training			
Ascorbic acid	$0.933 \pm 0.040$	$0.036 \pm 0.025$	$0.973 (n = 33)$
Uric acid	$0.873 \pm 0.046$	$0.058 \pm 0.025$	$0.961 (n = 33)$
Paracetamol	$0.942 \pm 0.031$	$0.032 \pm 0.021$	$0.984 (n = 33)$
Test			
Ascorbic acid	$0.919 \pm 0.081$	$0.065 \pm 0.041$	$0.953 (n = 15)$
Uric acid	$0.871 \pm 0.138$	$-0.008 \pm 0.086$	$0.868 (n = 15)$
Paracetamol	$0.895 \pm 0.105$	$0.082 \pm 0.071$	$0.921 (n = 15)$

## Conclusions

Although electronic tongues are not a new concept, an important new approach to pharmaceutical future works is presented in this work. Simultaneous quantification of three oxidizable analytes is carried out with good results by a simple electrode array and chemometric ANN modelling of the complex response. Simplicity is then the main achieved feature, although a complex data processing is needed, but this point is continuously improved and made cheaper everyday. Application to real blood samples or blood plasma would probably need sample pre-treatment, in order to minimize adsorption of fouling effects that could interfere in measurement stability in time. Automation with a sequential injection system is presented as an advantage for easy preparation of the huge amount of standards needed by the electronic tongue systems. Good modelling capacity has been achieved as well as acceptable prediction ability. Future works will try to determine the analysed compounds in real pharmaceutical samples.

*Acknowledgements.* Financial support for this work was provided by MEC (Madrid, Spain), through projects DPI2003-08229-C03-02, CTQ2004-08134, and by the Department of Universities and the Information Society (DURSI) from the Generalitat de Catalunya. We also thank Mr. Xavier Llopis for the measuring cell design.

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