



Potentiometric electronic tongue-flow injection analysis system for the monitoring of heavy metal biosorption processes

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ABSTRACT

An automated flow injection potentiometric (FIP) system with electronic tongue detection (ET) is used for the monitoring of biosorption processes of heavy metals on vegetable wastes. Grape stalk wastes are used as biosorbent to remove Cu^{2+} ions in a fixed-bed column configuration. The ET is formed by a 5-sensor array with Cu^{2+} and Ca^{2+} -selective electrodes and electrodes with generic response to heavy-metals, plus an artificial neural network response model of the sensor's cross-response. The real-time monitoring of both the Cu^{2+} and the cation exchanged and released (Ca^{2+}) in the effluent solution is performed by using flow-injection potentiometric electronic tongue system. The coupling of the electronic tongue with automation features of the flow-injection system allows us to accurately characterize the Cu^{2+} ion-biosorption process, through obtaining its breakthrough curves, and the profile of the Ca^{2+} ion release. In parallel, fractions of the extract solution are analysed by spectroscopic techniques in order to validate the results obtained with the reported methodology. The sorption performance of grape stalks is also evaluated by means of well-established sorption models.

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1. Introduction

The toxic effects of heavy metals in the environment are well known and governments introduce strict regulations with regard to metal discharge, mainly from industrial processes. Toxic metals are not easily biodegraded and enter in the food-chain where they can be accumulated on living tissues causing serious diseases. For these reasons, it becomes necessary to remove them from liquid wastes at least to the limit accepted by the regulations. The elimination of heavy metals in aqueous effluents frequently requires the use of complex and expensive technologies [1,2].

Recently, the ability of biological materials to fix metal ions has received considerable attention for the development of an efficient, clean and cheap technology for wastewater heavy metal removal. These materials represent a suitable alternative as compared to conventional sorbents frequently used (ion-exchange resins, activated carbon, etc.). Specially, their low cost makes their disposal feasible in contrast with an expensive regeneration. One of these sorbents, grape stalk wastes generated in the wine production process, resulted in a good sorption system for the elimination and

recovery of metal ions as Cu^{2+} , Cd^{2+} , Pb^{2+} , Ni^{2+} , Cr(VI) and Cr^{3+} . These studies have been carried out at laboratory scale, in both batch and continuous-flow methodologies [1–4].

First results obtained in batch experiments indicated that the sorption of heavy metals on grape stalks released alkaline and alkaline earth metals (K^+ , Mg^{2+} , Ca^{2+}), as well as protons [5], suggesting that ionic exchange is predominantly responsible for metal ion uptake.

Conventionally, analytical methods as flame atomic absorption spectroscopy (FAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and flow injection analysis with potentiometric detection (FIP) have been used in the monitoring of metal biosorption processes. The advantages of these methods are well known, however there are several drawbacks to consider: the spectroscopic methods use expensive equipment, are not well suited to on-line operation, require trained personal and, in many cases, a sample treatment is needed, affecting the analysis time. On the other hand, FIP is much user-friendly, produces data ideally at real time, permits automation and has a high sampling throughput. However, FIP depends of the quality and selectivity of the ion-selective electrodes used. Moreover, methods above may present some limitations on simultaneous or multiparametric analysis, an important aspect when working with real samples.

In the last years, the sensor's field has attempted the simultaneous analysis of species with a new strategy based on the use of multiple sensors showing cross-selectivity, in the approach known

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as electronic tongue (ET). According to the recent IUPAC definition [6] an ET is a “multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate data analysis—artificial neural networks (ANNs), principal component analysis (PCA), etc”. The purpose of the processing tool differs depending on the application; it can be to identify a chemical species or to determine its concentration without having to eliminate interferences or to quantify them at all [7].

Considering the number of works already published and the number of different laboratories working with their principles, electronic tongues and electronic noses, bio-inspired sensor analysis systems for liquid and gas determination respectively, can be qualified as a consolidated trend in the sensor's field, [8,9]. The applications of ET systems found in the literature are extended, especially for qualitative analysis (recognition, identification, classification) [7,10,11], even though quantitative analysis is becoming more important in the last few years [12,13], especially the resolution of multicomponent mixtures [14]. Thanks to these range of possibilities, ETs are starting to prove useful as quality control devices in the food industry/environment along with a few other applications such as water and process monitoring and clinical analysis [7]. The application of ETs in the simultaneous multi determination of heavy metals was successfully demonstrated in mine leachates [15], as well as more recently, in environmental field by using a light addressable potentiometric sensor (LAPS), prepared in an array format and furnished with differentiated selectivity chalcogenide thin films [16].

Sensor arrays have been integrated frequently in flow systems in order to automate the sample handling and the recording of the multichannel signals [7]. A recent review inspected in detail the use of electronic tongues as detection systems in flow systems, specifically by using flow injection analysis (FIA) principles as automation aid [17]. In these terms, a FIA system employing an array of chalcogenide glass sensors was described with the aim of determining heavy metals [18].

In the present work, the removal of Cu^{2+} ions from aqueous solutions onto grape stalk wastes in a fixed bed column is evaluated. The real-time monitoring of both the Cu^{2+} and the cation exchanged and released (Ca^{2+}) in the effluent solution is performed by potentiometric sensors by combining flow-injection techniques (FIP) and electronic tongue (ET) detection. Flow-injection potentiometric electronic tongues (ET-FIP) are a novel trend in the sensor field [7] in which the implementation of sensor arrays and FIP generates complex response peaks that can be processed with chemometric tools as a procedure to solve complex mixtures from chemical process analysis as, per example, metal biosorption from polluted effluents; the ET-FIP approach is intended for improving the performance of single sensor detection.

2. Materials and methods

2.1. Reagents and materials

The ionophores, calcium bis[4-(1,1,3,3-tetramethyl-butyl)-phenyl]-phosphate (CaBTMBPP), calcium-ionophore I (ETH 1001), tetrabenzyl pyrophosphate (TBPP), [2,2']-furdioxime monohydrate (FDMH); the plasticizers, dioctylphenylphosphonate (DOPP), 2-nitrophenyloctylether (NPOE), bis(1-butylpentyl)adipate (BBPA), dibutylphthalate (DBP); the additives, sodium tetrathenylborate (NaTPB) and potassium tetrakis(4-chlorophenyl)borate (KTpCIPB); and the polymer chromatographic grade poly(vinyl chloride) (PVC) were purchased from Fluka (Buchs, Switzerland).

Metal solutions were prepared by dissolving appropriate amounts of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}(\text{s})$ or $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}(\text{s})$ in deionised water (Milli-Q, Millipore; Molsheim, France). These reagents, tetrahydrofuran (THF) and metal standard solutions (1000 mg L^{-1} , used for Flame Atomic Absorption Spectroscopy, FAAS) were analytical grade and purchased from Merck (Darmstadt, Germany).

Grape stalk wastes generated in the wine production process (supplied by a wine manufacturer of Subirats, Penedès DO region, Barcelona, Spain), were rinsed three times with deionised water, dried in an oven at 110°C until constant weight, and finally cut and sieved for a particle size of 0.8–1.0 mm. Their properties as sorbents are in Ref. [19].

2.2. Membranes and electrodes

The ion-selective electrodes (ISEs) employed in the electronic tongue were all-solid-state tubular flow-through electrodes. Heterogeneous membranes based on a composite of $\text{Ag}_2\text{S}-\text{CuS}$ in epoxy resin were used in the preparation of the Cu^{2+} -selective sensors as is reported in the literature [19]. The Ca^{2+} -selective and the two generic sensors were prepared by using the corresponding ionophore in a PVC membrane, and constructed according to established procedures in our laboratories [20]. The composition of the sensor membranes used in the ET is listed in Table 1. All the flow-through tubular electrodes were placed inside a Perspex sandwich module, for their insertion in the flow system [26]. An Orion double-junction electrode (Model 90-02-00, Thermo Fisher Scientific, Beverly, MA, USA) was used as reference electrode.

2.3. Sorption experiments

All sorption experiments were conducted in duplicate in glass columns of 72 mm length and 10 mm internal diameter (Omnifit) and uniformly packed with 1.3 g of grape stalk waste (particle size of 0.8–1.0 mm) previously treated. During the column sorption operation, the aqueous metal solution containing approximately 35 mg L^{-1} of Cu^{2+} was pumped upwards through the column at a constant flow rate (30 mL h^{-1}). The sorption of Cu^{2+} ions from this solution involves ion-exchange processes where Ca^{2+} present in the grape stalk, are released. The influent solution in the column sorption consist in the solution of Cu^{2+} ion in deionised water, the effluent outlet of the column is composed by a variable content of calcium released from biosorbent as well as other ions (H^+ , K^+ , etc.) in lower amounts. The column effluent is pumped in fixed time intervals to the sensor array, which determines the concentration of calcium and copper in time. In order to compare with conventional analytical techniques, samples were collected from the outlet of the column by a fraction collector (Gilson FC204) at pre-set time intervals. Afterwards, metal concentrations in the influent and effluent solution were determined by flame atomic absorption spectrometry (FAAS, at a wavelength of 324.8 nm) using a Varian absorption spectrometer (Model 1275) and by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer model Optima 4300DV) for Cu^{2+} and Ca^{2+} , respectively. All experiments were performed at room temperature.

2.4. Breakthrough sorption capacity

The capacity at exhaustion q_{column} (mmol g^{-1}) is determined by calculating the total area below the breakthrough curve. This area represents the amount of solute sorbed by mass of solid in the sorption zone that goes from the breakpoint to exhaustion [3,4].

The breakthrough point is chosen arbitrarily at some low value, C_b (mmol L^{-1}); and the sorbent is considered to be essentially

Table 1
Composition of the sensor membranes employed in the array.

Sensor	PVC (%)	Plasticizer (%)	Ionophore (%)	Additive (%)	Reference
Ca1	33.3	<i>o</i> -NPOE (65.2)	Calcium-ionophore I, ETH 1001 (1.0)	NaTPB (0.5)	[21]
Ca2	29.8	DOPP (63.2)	Bis[4-(1,1,3,3-tetramethylbutyl)-phenyl]-phosphate, CaBTMBPP (7.0)	–	[22]
Generic1	34.5	BPA (63.2)	Tetrabenzyl pyrophosphate, TBPP (2.3)	KTpCIPB (X=0.4)	[23]
Generic2	32.0	DBP (62.0)	[2,2′]-Furildioxime monohydrate, FDMH (4.0)	KTpCIPB (2.0)	[24]
Cu	CuS (30.9%), Ag ₂ S (30.9%), araldite M (27.2%) and HR hardener (10.9%)				[25]

exhausted when the effluent concentration, C_x (mmol L⁻¹), reaches the 90% of C_0 (initial concentration of sorbate, mmol L⁻¹) [27,28].

$$q_{\text{column}} = \frac{\int (C_0 - C) dV}{m} \quad (1)$$

where C is the outlet metal concentration (mmol L⁻¹) and m is the mass of sorbent (g). The column sorption process requires prediction of the concentration–time profile or breakthrough curve for the effluent. Various mathematical models can be used to describe fixed bed sorption. One of the simplest and most widely used is the Thomas model [29]; this model allows determining the maximum concentration of solute in the solid phase as well as the sorption rate constant. The linearized form of the model is expressed as:

$$\ln \left(\frac{C_0}{C} - 1 \right) = \frac{k_{Th} q_0 m}{Q} - \frac{k_{Th} C_0 V_{eff}}{Q} \quad (2)$$

where k_{Th} is the Thomas rate constant (mL min⁻¹ mmol⁻¹), q_0 is the equilibrium metal uptake per gram of the sorbent (mmol g⁻¹), Q is the volumetric flow rate (mL min⁻¹), V_{eff} is the volume of effluent (mL) and m is the mass of sorbent inside the column (g).

The bed depth service timed (BDST) model, which was formulated by Hutchins [30,31] elucidates a relation between the service time and the packed-bed depth of the column and is expressed as:

$$C_0 t = \frac{N_0 h}{u} - \frac{1}{K} \ln \left[\frac{C_0}{C} - 1 \right] \quad (3)$$

where C_0 is the influent concentration (mmol L⁻¹), C is the effluent concentration at time t (mmol L⁻¹), K is the sorption rate constant (L (mmol min)⁻¹), N_0 is the sorption capacity (mmol L⁻¹), h is the bed depth of fixed-bed sorbent (cm), u is the linear flow rate (cm min⁻¹) and t is the service time to breakthrough (min).

The Yoon–Nelson model [32] is based on the assumption that the rate of decrease in the probability of sorption for each sorbate molecule is proportional to the probability of sorbate sorption and the probability of sorbate breakthrough on the sorbent [29,32]. The linearized model for a single component system is expressed as:

$$\ln \frac{C_t}{C_0 - C} = k_{YN} t - \theta_r k_{YN} \quad (4)$$

where k_{YN} is the rate constant (per min) and θ_r is the time required for 50% sorbate breakthrough (min).

If the dynamic behaviour of a fixed-bed sorption column can be predicted by the above-described models, the Marquardt's percent standard deviation (MPSD) indicates an estimation of error between the experimental and theoretical values of C/C_0 used for plotting the breakthrough curve; the MPSD can be calculated by using the following equation [33]:

$$\text{MPSD} = 100 \cdot \sqrt{\frac{1}{N-P} \sum \left[\frac{(C/C_0)_{\text{exp}} - (C/C_0)_{\text{theo}}}{(C/C_0)_{\text{exp}}} \right]^2} \quad (5)$$

where N is the number of data points and P is the number of parameters (or the degrees of freedom of the system).

2.5. Electronic tongue-flow injection experiments (ET-FIP)

Five flow-through tubular electrodes were used in the developing of the ET-FIP system (see Table 1). Two sensors based on different ionophores were selective to Ca²⁺ ions (Ca1 and Ca2), another sensor was selective to Cu²⁺ ions (Cu), and two generic sensors were based on different ionophores (Generic 1 and Generic 2), the first a Pb²⁺ ISE with residual response to Ca²⁺ and Cu²⁺, and the second a Ca²⁺ ISE with strong interference of Cu²⁺.

In these experiments, the same manifold of the computer controlled-flow injection potentiometric system developed by Florido et al. [19] as well as the hardware, software, monitoring procedure, optimized flow parameters and other characteristics, were used.

The carrier solution used in all experiments consisted on sodium nitrate solution 0.2 mol L⁻¹, used as ionic strength adjustor, plus equimolar aqueous solution of Cu²⁺ and Ca²⁺ nitrates with total concentration of 2.0 × 10⁻⁶ mol L⁻¹, this background level of the two target ions was added in order to improve recovery of the baseline.

The nonlinear response of the sensors caused by the interference effect was modelled adequately by ANN [34]. For training purpose, the patterns used in the ET-FIP consisted on 36 different standard solutions (mixtures of Ca²⁺ and Cu²⁺ ions) prepared by dilution from a 1 mol L⁻¹ stock solution. From these, an independent subset of 10 analogue solutions was employed for evaluating modelling performance of the developed approach. The concentration levels ranged from 0 to 60 mg L⁻¹ (0–0.0015 mol L⁻¹) for Ca²⁺ and 96 to 0 mg L⁻¹ (0.0015–0 mol L⁻¹) for Cu²⁺ ions. ANN calculations were done in MATLAB 7.0 (MathWorks, Natick, MA) using its Neural Network Toolbox (v.4.0).

3. Results and discussion

The previous on-line computer controlled-flow injection potentiometric system [19] was improved by introducing as detection system an electronic tongue based on an array of chemical sensors for the monitoring of metal-biosorption processes. The array consisted in 5 different selective and non-specific tubular potentiometric sensors, based on heterogeneous and polymeric membranes. The combination of the electronic tongue and the on-line computer-controlled flow injection potentiometric system (ET-FIP) should provide an analytical methodology with important advantages in the simultaneous monitoring of metal ions during biosorption processes in grape stalk wastes.

3.1. Sensor characterization

First experiments were carried out in order to characterize the main analytical features of the prepared potentiometric sensors. The results for these evaluations are summarized in Table 2. All electrodes presented Nernstian responses for the corresponding

Table 2
Response characteristics of the ISEs used in ET-FIP.

Sensor	Sensitivity to Ca ²⁺ (mV/dec) ^a	Sensitivity to Cu ²⁺ (mV/dec) ^a	Concentration range (mol L ⁻¹)	PDL (mol L ⁻¹) ^a	Interference ^b	
					log $K_{Ca,Cu}^{pot}$	log $K_{Cu,Ca}^{pot}$
Ca1	29.8 ± 0.9	22.6 ± 0.8	7.5 × 10 ⁻⁷ –6.5 × 10 ⁻²	(4.2 ± 0.6) × 10 ⁻⁷	-3.2 ± 0.2	-
Ca2	30.1 ± 1.1	17.8 ± 0.9	5.7 × 10 ⁻⁶ –0.5 × 10 ⁻²	(1.3 ± 0.7) × 10 ⁻⁶	-2.5 ± 0.3	-
Cu	17.6 ± 1.2	30.4 ± 1.3	8.6 × 10 ⁻⁶ –1.2 × 10 ⁻²	(2.0 ± 0.5) × 10 ⁻⁶	-	-3.5 ± 0.2
Generic1	25.8 ± 1.2	28.0 ± 1.5	6.8 × 10 ⁻⁶ –1.3 × 10 ⁻²	(3.5 ± 0.8) × 10 ⁻⁶	2.2 ± 0.2	-2.5 ± 0.3
Generic2	29.8 ± 0.8	21.2 ± 1.6	8.7 × 10 ⁻⁶ –2.1 × 10 ⁻²	(4.1 ± 0.9) × 10 ⁻⁶	-2.3 ± 0.1	1.9 ± 0.2

^a n = 10.

^b n = 4.

primary ion, with slopes around +29 mV/dec, as expected for divalent cations. The values of the practical detection limits (PDL) were around to 10⁻⁶ mol L⁻¹ for all ISEs except for Ca1 sensor which presented a PDL of 4.2 ± 0.6 × 10⁻⁷ mol L⁻¹. The electrodes were practically used every day during 3 months in different experiments; in this period the stability of the electrodes was studied and the values of slope and PDL for all sensors were similar to initial values, with standard deviations (for 20 determinations) lower than ±1.6 mV/dec and ±0.9 × 10⁻⁶ mol L⁻¹ for slope and PDL, respectively.

The presence of cross-response is a pre-requisite characteristic of ISEs to be considered when building an ET [6,35]. In order to estimate the cross-selectivity of the sensors used in this work, the potentiometric selectivity coefficients were determined according to the IUPAC recommended fixed interference method [36] (FIM). According to the values of the selectivity coefficients obtained, all sensors showed certain cross response to the target ions. This fact can be seen in Table 2 and Fig. 1 (fiagram obtained during calibration of the five flow-through sensors employed in the ET at different concentration levels of Cu²⁺ ions), where even the best Ca²⁺ ISE employing ionophore ETH1001 displays response to Cu²⁺ when Ca²⁺ is absent.

The concentration range of linear response was determined with the objective to establish the concentration levels for the calibration model and quantify the samples. For all electrodes, the range values were between 8.7 × 10⁻⁶ and 0.5 × 10⁻² mol L⁻¹, except for Ca1 sensor that showed values between 7.5 × 10⁻⁷ and 6.5 × 10⁻² mol L⁻¹.

3.2. Optimization of the electronic tongue-flow injection potentiometric system

The complete optimization of FIA parameters and manifold for a single sensor were performed by Florido et al. [19]. In the present study, where an array with five sensors is involved, only the influences of flow rate and injection time were verified again for all sensors of the ET. Two flow rates (1.0 and 2.0 mL min⁻¹) were studied at two concentration levels (1.0 × 10⁻⁴ and 1.0 × 10⁻³ mol L⁻¹) for all ISEs of the ET. Higher peaks heights were obtained at 2.0 mL min⁻¹ for both concentration levels, which coincides with previous work [19]; good mixing characteristics and reduced axial diffusion of injected species may be the explanation for this observation.

The sample and standards injected volume introduced in the ET-FIP system was adjusted by controlling the opening time of the solenoid valves. This time was optimized by studying the response into the system of a 10⁻⁴ mol L⁻¹ Ca²⁺ solution, at different valve opening times: 20, 40, 60, 80, 100, 140 and 180 s. Fig. 2 shows the response obtained for the Ca1 sensor. It can be seen that 60 s was the minimum injection time that gives sharp peaks and, at the same time its peak height is approximately the 95% of the steady-state potential. For these reasons, 60 s was selected as the injection time. The same value was obtained with the other four ISEs of the array. This activation time corresponds to an injected sample volume of 2000 μL.

In order to verify the correct operation of all parts of the proposed ET-FIP system, Ca²⁺ and Cu²⁺ standard solutions at same concentration (5.5 × 10⁻⁴ mol L⁻¹) were prepared and introduced individually into the system from different inlets (sample or standards). Metal concentrations were determined from previous calibration curves, in flow conditions, and the precision values

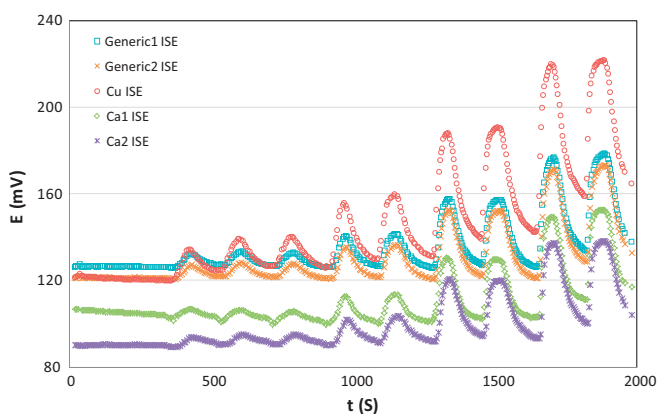


Fig. 1. Diagrams obtained during calibration of the flow-through sensors for different concentration levels of Cu²⁺ ions (10⁻⁵, 10⁻⁴, 10⁻³ and 10⁻² mol L⁻¹); two consecutive peaks for all concentration levels except for 10⁻⁵ mol L⁻¹.

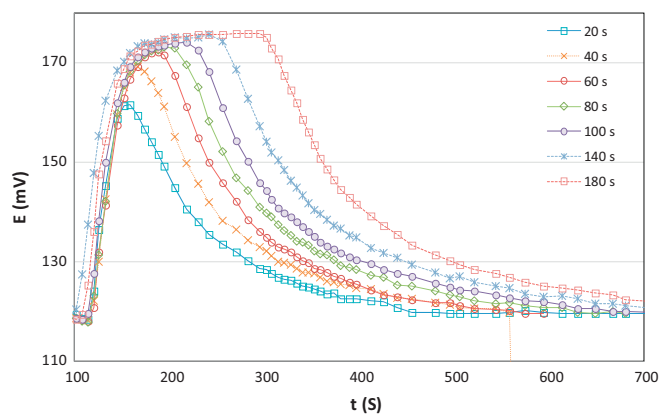


Fig. 2. Effect of the injection time (injected sample volume) on the peak responses of the Ca1 sensor. Peaks were obtained for Ca²⁺ standard solution (1.0 × 10⁻⁴ mol L⁻¹) and at a constant flow rate of 2.0 mL min⁻¹. The potential at the stationary state was 176 ± 1 mV.

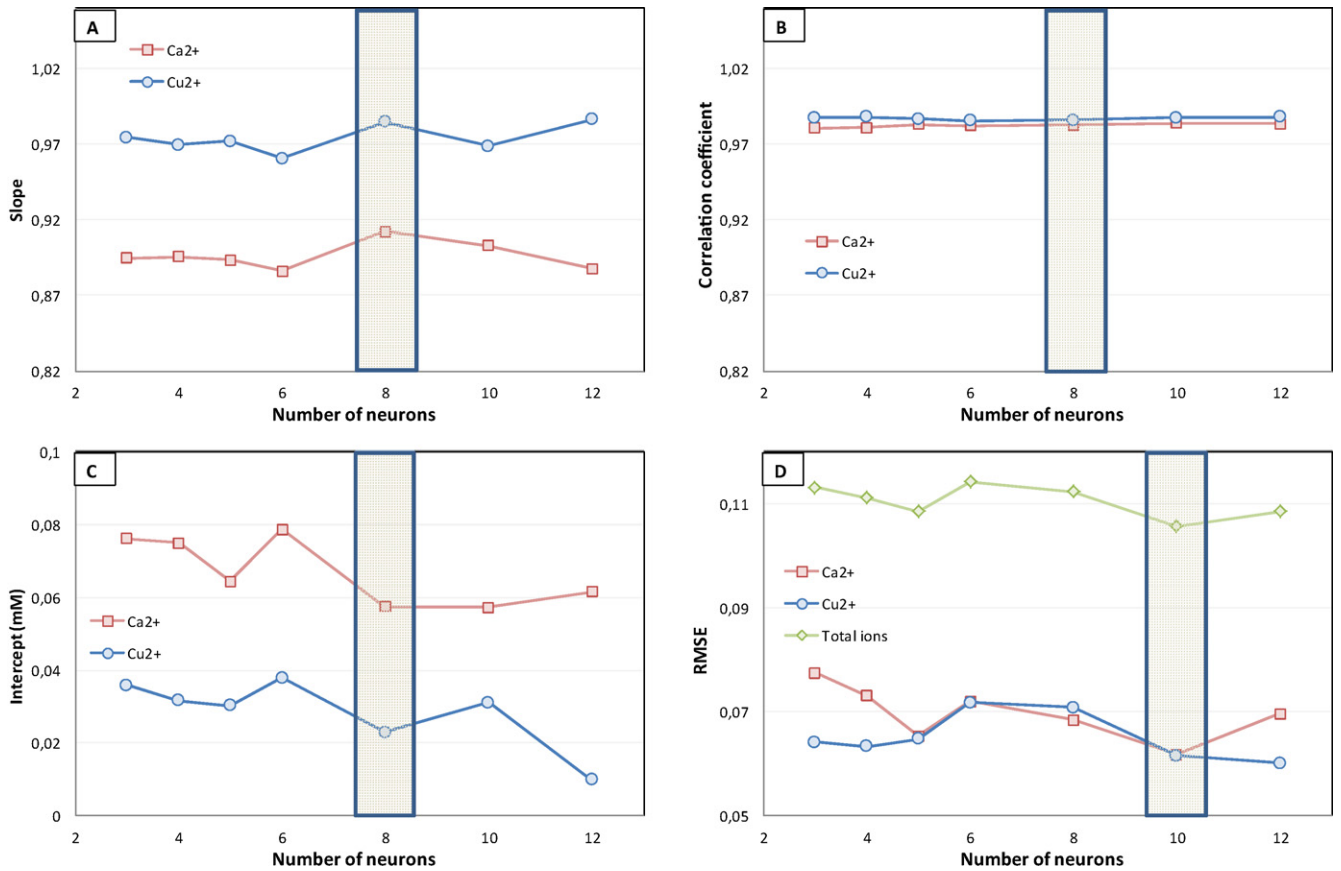


Fig. 3. Selection of the optimal number of neurons in the hidden layer for the ANN model considering: (A) slopes; (B) correlation coefficients; (C) intercepts and (D) RMSE values for the comparison regression between obtained vs. expected results.

obtained were 4.9% RSD and 4.5% RSD for Ca^{2+} and Cu^{2+} , respectively ($n = 10$, repetitivity calculations).

3.3. Building of the ANN response model

Artificial neural networks (ANNs) were used as the mathematical method of data processing to build the response model in this work. A total of 36 solutions, mixtures of Ca^{2+} and Cu^{2+} ions, were prepared according to a factorial design scheme (6 levels, 2 factors) in order to obtain the model response. The set of standard solutions were split into two subsets: a training subset containing 26 samples, used to build the response model, and a test subset containing 10 samples, used to evaluate the model predictive

ability. The selection is done randomly [37] and avoiding samples with maximums and minimums in the testing subset, in order to eliminate any extrapolation.

Before the optimization process, the following characteristics of the ANN were fixed, as accumulated experience from our laboratories [38]: the type of network (feedforward, backpropagation with multiple output), the training algorithm (Bayesian regularization), the training parameters: learning rate ($\alpha = 0.1$) and momentum ($\beta = 0.4$), the use of a single hidden layer, 5 neurons in the input layer (one per each ISE peak heights) and 2 neurons (one per each analyte concentration) in the output layer. In order to get a certain level of confidence in the final results, other configuration parameters were optimized such as the number of neurons in the hidden

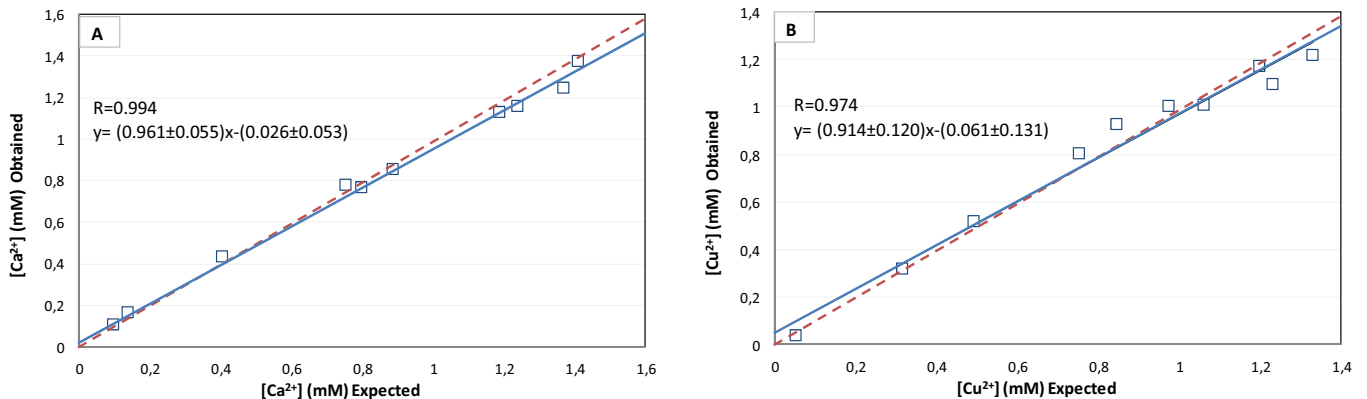


Fig. 4. Modelling performance achieved for the optimized ANN and the external test set: (A) response to Ca^{2+} , (B) response to Cu^{2+} . The dashed line corresponds to ideality, and the solid line is the regression of the comparison data.

layer, and the transfer functions in the hidden and output layers (linear or non-linear). The selection of the best combination of the ANN parameters was performed based on the root mean square error (RMSE) and prediction abilities obtained by the calculated vs. expected comparison graphs for the two metals.

Fig. 3 shows a fragment of the optimization process in order to select the optimal number of neurons for certain combination of transfer functions. After comparison of all results, the following characteristics of network were considered: 8 neurons in the hidden layer, and *tansig* (hyperbolic tangent sigmoid) and *purelin* (linear) as transfer functions in the hidden and output layers, respectively.

Fig. 4 illustrates the correlation between the obtained (y) and expected (x) concentration values of the two individual ions for the external test subsets. As can be seen, the model prediction is fairly good for both ions, with correlation coefficients, slopes and intercept values close to the theoretical ones, illustrated as the diagonal line. At the same time, the prediction values (RMSE) were in agreement with the above facts. In the correlation analysis (all confidence intervals were calculated at the 95% confidence level), the dispersion and the limit of confidence of the slope and intercept for the Cu^{2+} response were greater than for the Ca^{2+} one. This could be attributed to the fact that the sensor array in the ET is formed for two different Ca^{2+} electrodes and only one Cu^{2+} membrane, a fact giving a richer, more differentiated response for the former.

3.4. Evaluation of ET-FIP monitoring of ion-exchange processes involved in metal-biosorption

Finally, a fixed-bed column filled with grape stalk wastes was coupled to the ET-FIP system in order to study the removal of Cu^{2+} ions from the aqueous solution. Thus, the Cu^{2+} sorption onto the biosorbent, and the subsequent release of Ca^{2+} ions were monitored by ET-FIP system. The set of responses obtained from the sensor array was processed by employing the ANN model previously optimized, obtaining in this way the two concentrations sought.

Biosorption experiments were performed at a similar way as reported in [19]. The only change in the procedure was that the training was performed immediately after the biosorption process to avoid variability caused by time. In addition and in order to counteract any possibility of sensor drift, a standard solution was introduced into the system every 4 h during the biosorption process in the column and during the training and validation process of the electronic tongue. Considering that a complete biosorption experiment and the training and validation process in our conditions took about 26 and 8 h of monitoring respectively, it is important to point out that the drift for each individual sensor was examined carefully. The maximum potential drifts obtained during the experiments are around $\pm 0.2 \text{ mV h}^{-1}$, this signal change is not considered relevant for the study.

Fig. 5 shows the breakthrough curves for the Cu^{2+} -biosorption process and the release of Ca^{2+} ions from the grape stalks, relative to volume of effluent pumped. These curves were also plotted by the conventional method of calculating the ion concentration from the calibration curve of the corresponding individual ISE (without interference correction) and by using the cross-selectivity involved in the ET-FIP system. Moreover and in order to compare with established analytical procedures, Fig. 5 also shows the copper breakthrough curves obtained by FAAS and the profile of the calcium concentration values, quantified by ICP-OES.

As can be seen, there are some discrepancies between the curves when considering the conventional method (individual ISE without interference correction) and by using the ET-FIP system. The main differences between the methods were obtained in the range between 100 and 400 mL of effluent pumped in the case of copper

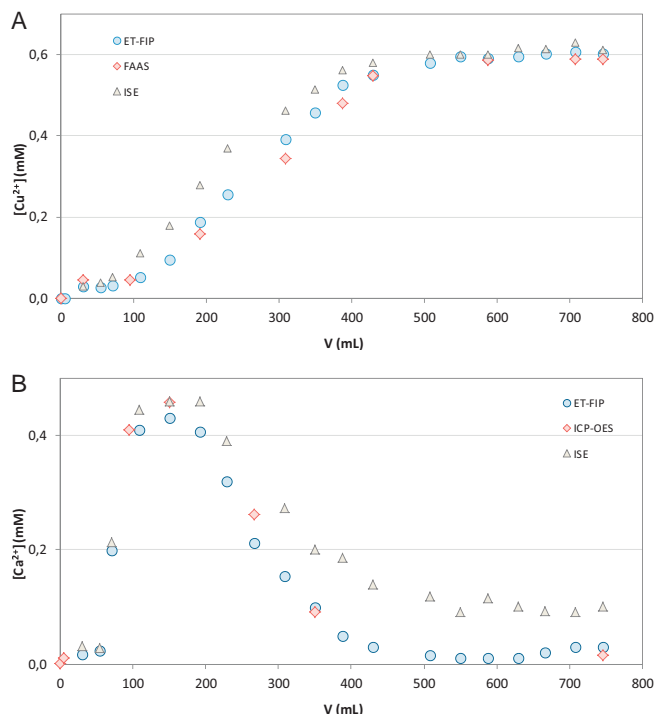


Fig. 5. The breakthrough curve for the sorption of Cu^{2+} onto grape stalks obtained by the on-line ET-FIP system, by ISE and by FAAS (A); and profile of the Ca^{2+} ion obtained by the on-line ET-FIP system, by ISE and by ICP-OES (B).

curves, and from the 220 mL to the end of experiment for calcium profiles. The results of both methods were compared statistically by the Student's paired sample t test; the calculated t statistics are: $t = 8.17$ for calcium determination and $t = 4.77$ for copper determination. In both cases, the t statistics are greater than the critical tabulated value of $t^* = 2.10$ and 2.11 for calcium and copper with 18 and 17 degrees of freedom respectively and 95% confidence level. This statistical analysis indicates that there are significant differences between results obtained with the conventional method of calibration curve for individual ISE and those of the electronic tongue, in detriment of the direct potentiometric method. The relative error values were calculated between results from the two compared methods and corroborated the last observation. In the severe interference ranges mentioned above, relative errors reached values of 39–208% and 12–101% were obtained for calcium and copper respectively. Fig. 5 can also be employed to compare the copper breakthrough curves obtained by the ET-FIP system and by FAAS and the profile of the calcium concentration quantified by ET-FIP system and by ICP-OES. In this case, the statistical comparison yielded a calculated value for calcium determination of $t = 2.27$, lower than the tabulated value for 7 degrees of freedom ($t^* = 2.36$), and $t = 0.65$ for copper, also lower than the tabulated value for 4 degrees of freedom ($t^* = 2.78$). Correlations obtained were 0.9951 ($n = 8$) and 0.9945 ($n = 5$) for calcium and copper determination, respectively. As this statistical test demonstrates, the results obtained by the proposed ET-FIP system showed an excellent agreement with those determined by reference methods (the spectroscopic methods) for both ions. This better correlation could be explained by the fact that the electronic tongue takes profit of cross-sensitivity and thus it considers and compensates the effect of interferences on the electrode response. It is then demonstrated that ET based on a sensor array reports more accurate results than those obtained by using individual ISEs, when directly used without performing any chemical treatment to mask or to eliminate interfering effects of the sample or other ions in the procedure. In

Table 3Comparison of Thomas, BDST and Yoon–Nelson model and experimental parameters for Cu²⁺ sorption onto grape stalk wastes.

	Model		Experimental	
Thomas	q_0 (mmol g ⁻¹)	0.112	q_0 (mmol g ⁻¹)	0.116
	K_{Tn} (mL mmol ⁻¹ min ⁻¹)	15.63		
BDST	N_0 (mmol L ⁻¹)	27.60	N_0 (mmol L ⁻¹)	28.75
	K_{BDST} (ML mmol ⁻¹ min ⁻¹)	15.63		
Yoon–Nelson	θ_r (min)	502.6	θ_r (min)	509.7
	K_{YN} (min ⁻¹)	0.009		
r^2		0.97		
MPSD		9.67		

fact and also deduced from Fig. 5, one can observe that Cu²⁺ ions affect more the response of the Ca electrodes than Ca²⁺ ions the Cu sensor response, when considered as individual ISEs. This effect is in agreement to the values of the potentiometric selectivity coefficients presented in Table 2, where the interference of Cu²⁺ shows to be somewhat stronger.

3.5. Modelling the ion exchange mechanism

In order to characterise the performance of the grape stalk wastes in fixed bed column for Cu²⁺ sorption, the experimental data obtained by the ET-FIP technique was fitted to the Thomas, BDST and Yoon–Nelson models. The breakthrough and the exhaustion volumes observed experimentally are reached after 71 and 420 mL, respectively. The sorption parameters obtained from slopes and intercepts of linear plots of Eqs (2)–(4) [28] are summarized in Table 3 and compared to the experimental data. The sorption parameters obtained were in concordance to the experimental breakthrough data. Thus, the sorption capacity q_0 reported by the Thomas model was 0.112 mmol L⁻¹ while the experimental value was 0.116 mmol L⁻¹ representing less than 3.7% of error. In the same sense, the sorption capacity of bed volume N_0 reported by the BDST model and the time required for 50% sorbate breakthrough θ_r reported by the Yoon–Nelson model were close to the experimental values as shown in Table 3. Furthermore, the MPSD value indicates a good fit of the Thomas, BDST and Yoon–Nelson models to Cu²⁺ sorption experimental data.

Previous studies showed grape stalk wastes as potential sorbent for metal removal from aqueous solution in batch experiments [5,39]. In these works, sorption of Cu²⁺ on grape stalks showed a significant release of Ca²⁺ and alkaline metals (K⁺, Mg²⁺) as well as protons, indicating that ionic exchange is predominantly the responsible mechanism for metal ion uptake. In this study, the release of Ca²⁺ was monitored in the effluent solution in order to evaluate the sorption behaviour of the exchanged cation Cu²⁺. As can be seen in Fig. 6, the sorption of Cu²⁺ ions from the solution involves ion-exchange processes where Ca²⁺ ions present in the original grape stalk structure, are released. Thus, after the

breakthrough point (around 70 mL), the concentrations of Ca²⁺ reached its maximum with a continuous increase of the Cu²⁺ concentration. The consequent decrease on the Ca²⁺ concentrations indicates the exhaustion of the exchange capacity of the sorption column (the exhaustion point is around 420 mL). The total amount of metal bound and released were 0.233 and 0.147 meq g⁻¹ for Cu²⁺ and Ca²⁺, respectively. These values were quite close to those previous obtained in batch experiments [39].

4. Conclusions

An electronic tongue flow-injection potentiometric system has been proposed for the monitoring of the ion-exchange processes involved in the sorption of Cu²⁺ onto grape stalks and release of Ca²⁺ ions. The results obtained showed an excellent agreement with those determined by reference methods for both ions. This better correlation could be explained by the fact that the electronic tongue takes profit of cross-sensitivity and thus it considers and compensates the effect of interferences on the electrode response. It demonstrates that ET based on a sensor array reports more accurate results than those obtained by directly using individual ISEs, with relative error of 86.0% and 74.0% for Ca²⁺ and Cu²⁺ respectively for conventional calibration method and 4.1% and 4.4% for Ca²⁺ and Cu²⁺, respectively, for the proposed ET. Moreover, it has been demonstrated that with the proposed ET-FIP approach, a quantitative multi-determination of a short number of chemical species is easily attainable. However, the developed system offers a wide range of possibilities and the conjunction of flow-injection potentiometry and electronic tongues has appeared as an integrated option for the automatic monitoring of laboratory or pilot-plant processes, resulting in a versatile and expandable instruments. In the illustrated case, a precise, interference compensating and completely automatic procedure was attained to characterize without any manual intervention the sorption performance of the grape stalks as a biosorbent material.

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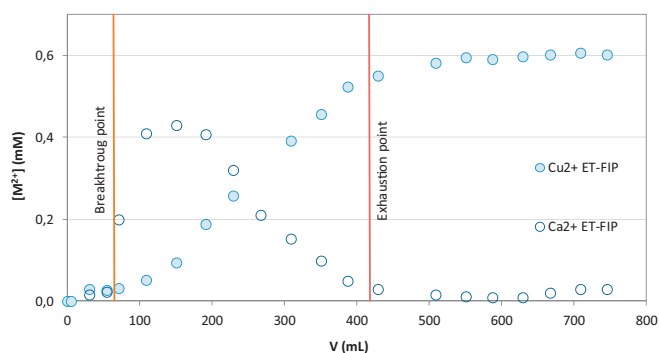


Fig. 6. The breakthrough curve for the sorption of Cu²⁺ ion onto grape stalks and profile of the Ca²⁺ ion, both obtained by the on-line ET-FIP system.

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