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PAPER

Resolution of phenolic antioxidant mixtures employing a voltammetric bio-electronic tongue†‡

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This work reports the application of a Bio-Electronic Tongue (BioET) system made from an array of enzymatic biosensors in the analysis of polyphenols, focusing on major polyphenols found in wine. For this, the biosensor array was formed by a set of epoxy–graphite biosensors, bulk-modified with different redox enzymes (tyrosinase and laccase) and copper nanoparticles, aimed at the simultaneous determination of the different polyphenols. Departure information was the set of voltammograms generated with the biosensor array, selecting some characteristic features in order to reduce the data for the Artificial Neural Network (ANN). Finally, after the ANN model optimization, it was used for the resolution and quantification of each compound. Catechol, caffeic acid and catechin formed the three-analyte case study resolved in this work. Good prediction ability was attained, therefore allowing the separate quantification of the three phenols with predicted *vs.* expected slope better than 0.970 for the external test set ($n = 10$). Finally, BioET has been also tested with spiked wine samples with good recovery yields (values of 104%, 117% and 122% for catechol, caffeic acid and catechin, respectively).

Introduction

Polyphenols are a group of naturally occurring compounds that can be defined as molecules with more than one hydroxyl group on one or more phenol units per molecule. Most of these compounds are powerful antioxidants needed for the functioning of plant cells, which can be found in fruits and vegetables such as apples and onions, or beverages such as tea or wine.[1]

There is evidence that wine has antioxidant properties, that these are due to polyphenolic components, and that wine without these components loses these properties.[2] The total content of polyphenols in wines is directly correlated with their antioxidant capacity;[3,4] its exact composition is conditioned by the grape variety, geographical location and winemaking technology. The total concentration of polyphenols in wine varies between 1.80 and 4.06 g l^{-1} equivalents in gallic acid for red wines and between 0.16 and 0.33 g l^{-1} for white wines; being gallic acid, catechin, epicatechin, *p*-coumaric acid, caffeic acid and catechol the major polyphenols found in wine.[5–7] However, individual polyphenols concentration values are much lower than the total ones stated above, *e.g.* caffeic acid with a content of 5–13 mg l^{-1} (27–72 μM) for red wines and 1–4 mg l^{-1} (5–22 μM) for white wines or catechin with a content of 120–390 mg l^{-1} (413–1343 μM) for red wines and 16–46 mg l^{-1} (55–158 μM) for white wines are some of the typical contents.

One the one hand, several methods to quantify total phenols and polyphenols have been described in the literature.[8] Common determinations of polyphenols include procedures such as the reaction with 4-aminoantipyrine,[9,10] Folin–Ciocalteu reagent[11] or cyclic voltammetry analysis;[12] all these methods yield a total phenol content value, and therefore do not allow for the discrimination between individual phenols. On the other hand, there are techniques such as HPLC[13–16] or GC[17,18] which allow individual identification of polyphenols, but these require specific equipment, laboratory conditions and/or trained personnel. Finally, we cannot forget the use of amperometric biosensors which represent an attractive alternative to classical analytical methods for the detection of polyphenolic compounds; for this aim, biosensors incorporating enzymes such as tyrosinase,[19,20] laccase[21,22] or peroxidase[23,24] have been employed. Even, the immobilization of two enzymes in the same biosensor has been reported.[25,26]

In the recent years, hybrid intelligent analytical systems have started to play an important role in the food industry; one example of this is what is called an Electronic Tongue (ET).[27–29]

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‡ Electronic supplementary information (ESI) available: Table containing detailed composition of each electrode, BioET response towards individual components of the mixture and detailed results of the ANN optimization. See DOI: 10.1039/c1an15456g

These biomimetic systems have provided alternatives to many classical methods in the food analysis field.[30–32] ETs have been already used for the detection of phenols in water from a number of impedimetric sensors interrogated at specific frequencies,[33] or even to carry out their quantification from the overlapped voltammetric signal obtained with a carbon electrode[34] or with a tyrosinase amperometric biosensor.[35] Even, the phenolic content of extra virgin olive oils was predicted from an array of chemically modified voltammetric electrodes;[36] and the generic pollution indicator, phenol index, has been deducted from the signal obtained with a laccase amperometric biosensor.[37]

The ET is a new trend in the chemical analysis field: this approach uses a system formed by an array of sensors capable of giving a wide and complete response of the analyzed species, plus a chemometric processing tool, capable of extracting meaningful data from the complex readings provided by the sensor array.[38–40] In this way, Artificial Neural Networks (ANNs), broadly used in chemical data-mining, are usually employed in electronic noses and electronic tongues.[41,42] This is because ANNs are powerful modelling tools for deriving the meaning from complex or imprecise data; their superior performance makes them irreplaceable in chemometrics tasks such as multivariate calibration or pattern recognition.[43,44]

Thus, the main contribution presented in this work is the use of an array of voltammetric enzyme biosensors. This is why the approach can be named Bio-Electronic Tongue (BioET). This approach has been rarely used in the literature, probably because the processing of a set of voltammetric signals is a case of high dimensionality and complexity, especially if ANNs are to be used. Similar cases required a multi-way treatment[45] or a pre-processing step for data reduction, such as Feature Extraction, Principal Component Analysis (PCA) or Discrete Wavelet Transform (DWT).[46] This data pre-processing, needed to make this case compatible with ANNs, permits one also to gain advantages in training time, to avoid redundancy in input data and to obtain a model with better generalization ability.

The BioET employed here is formed by an array of amperometric enzymatic biosensors based on bulk-modified biocomposites for the simultaneous determination of catechol, caffeic acid and (±)-catechin, in the concentration range found in wines. Using the combined electrochemical responses obtained from the set of biosensors' voltammograms to complement the departure information, performing specific feature extraction and employing an ANN model, it was possible to carry out the resolution of the mixture both in synthetic samples and in spiked wine samples, achieving their correct quantification.

Experimental

Apparatus

Electroanalytical experiments were carried out at room temperature (25 °C) using a 6-channel AUTOLAB PGSTAT20 (Ecochemie, Netherlands) in a multichannel electrode configuration under quiescent conditions. A double junction electrode Ag/AgCl Orion 900200 (Thermo Electron Corporation, Beverly, MA, USA) and a platinum-based electrode (Crison 52-67, Barcelona, Spain) were used as reference and auxiliary electrodes respectively.

Reagents and solutions

All reagents used were analytical grade and all solutions were prepared using deionised water from a Milli-Q system (Millipore, Billerica, MA, USA). Tyrosinase from mushroom (EC 1.14.18.1, 4276 U mg^{-1}), laccase from *Trametes versicolor* (EC 1.10.3.2, 21 U mg^{-1}), catechol, caffeic acid, (±)-catechin hydrate, potassium dihydrogenphosphate and potassium monohydrogenphosphate were purchased from Sigma-Aldrich (St Louis, MO, USA). KCl was purchased from Merck KGaA (Darmstadt, Germany). Copper nanoparticles (50 nm) were provided by Nanotek SA (Argentina).

Determinations were carried out in a 0.1 M phosphate buffer at pH 6.50, prepared in KCl 0.1 M, for ensuring a high electrical conductivity. 1 mM phenolic stock solutions were prepared by dissolving the right amount of pure substance (catechol, caffeic acid and (±)-catechin) into the buffer solution. After preparation of the stock solutions, training and testing samples were prepared by taking different microvolumes of phenol stock solutions and adjusting to a final volume of 25 ml. Concentrations ranged from 0 to 200 μM for each polyphenol (Fig. 1); 27 (73% of the total) samples distributed in a cubic design[47] were used for the training set and 10 additional samples, distributed randomly along the experimental domain, were used for the external test set (27% of the total).

Wine spiked samples

In order to perform the application with real samples and to verify if the wine matrix represents a problem for the resolution and quantification of individual polyphenols, 10 extra samples were prepared adding different quantities of a stock solution of each polyphenol to a standard table wine. As the ones prepared for the external test set, its concentration was ranged and distributed randomly along the experimental domain (Fig. 1).

Voltammetric sensor array

An array of 4 different graphite–epoxy voltammetric biosensors was prepared following the conventional methodology

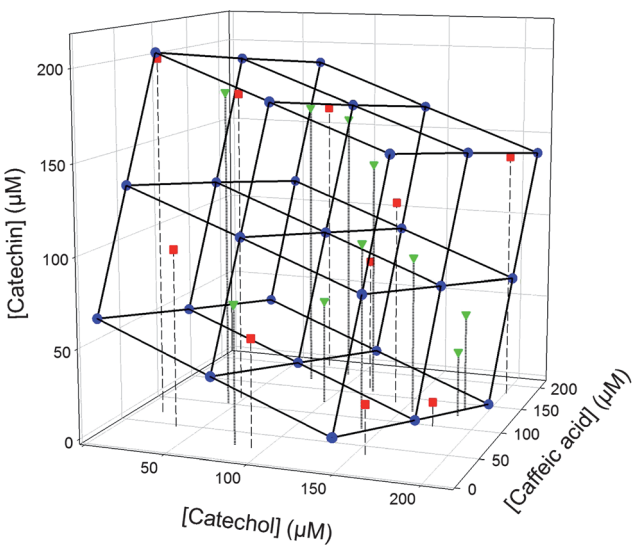

Fig. 1 Experimental design for the training subset (●), the external test subset (▼) and the spiked wine samples (■).

previously established in our research group.[48] First, resin Epo-Tek H77 (Epoxy Technology, Billerica, MA, USA) and its corresponding hardener compound were mixed in the ratio 20 : 3 (w/w). Then, each electrode was prepared adding 15% of graphite (w/w) and 2% of either the enzyme (tyrosinase or laccase) or the modifier (w/w) (copper nanoparticles) into the epoxy resin before hardening[49]—one component per electrode, and the fourth electrode without any modifier (detailed composition of each sensor is summarized in Table S1 included in the ESI‡). Afterwards, the biocomposite was manually homogenised for 60 min. Finally, the biocomposite paste electrode was allowed to harden for 7 days at 40 °C. The electrode surface was then polished with different sandpapers of decreasing grain size, with a final electrode area of 28 mm^2.

Tyrosinase catalyzes the hydroxylation of monophenols to catechols, which in turn are further oxidized to *o*-quinones, both using molecular oxygen; then *o*-quinones produced in the enzymatic reaction are electrochemically reduced to *o*-diphenols, resulting in recycling of the diphenols at the applied negative potential. Laccase catalyzes the oxidation of phenols giving phenoxyradical species which are converted to quinones in the second stage of the oxidation; then as in tyrosinase, these quinones can be electrochemically reduced again to phenols. Finally, copper-nanoparticles were chosen given that both tyrosinase and laccase are copper-containing enzymes and it was thought that they could also have some catalytic effect as demonstrated with the results obtained.

Procedures

The measurement cell was formed by the 4-sensor voltammetric array plus the reference and auxiliary electrodes. Working electrodes were cycled 3–5 times in buffer solution in order to get stable voltammetric responses before performing the measurements with prepared samples. The potential was swept between +0.8 V and −0.4 V *vs.* Ag/AgCl, with a scan rate fixed at 100 mV s^{-1} and a step potential of 9 mV. No pre-treatment of the sample was performed; and all experiments were carried out without performing any physical surface regeneration of the working electrodes. In order to prevent the accumulative effect of impurities on the working electrode surfaces, an electrochemical cleaning stage was done between each measurement applying a conditioning potential of +1.0 V for 40 s after each experiment in a cell containing 10 ml of distilled water.[50]

Data processing

Chemometric processing was done by specific routines in MATLAB 7.0 (MathWorks, Natick, MA) written by the

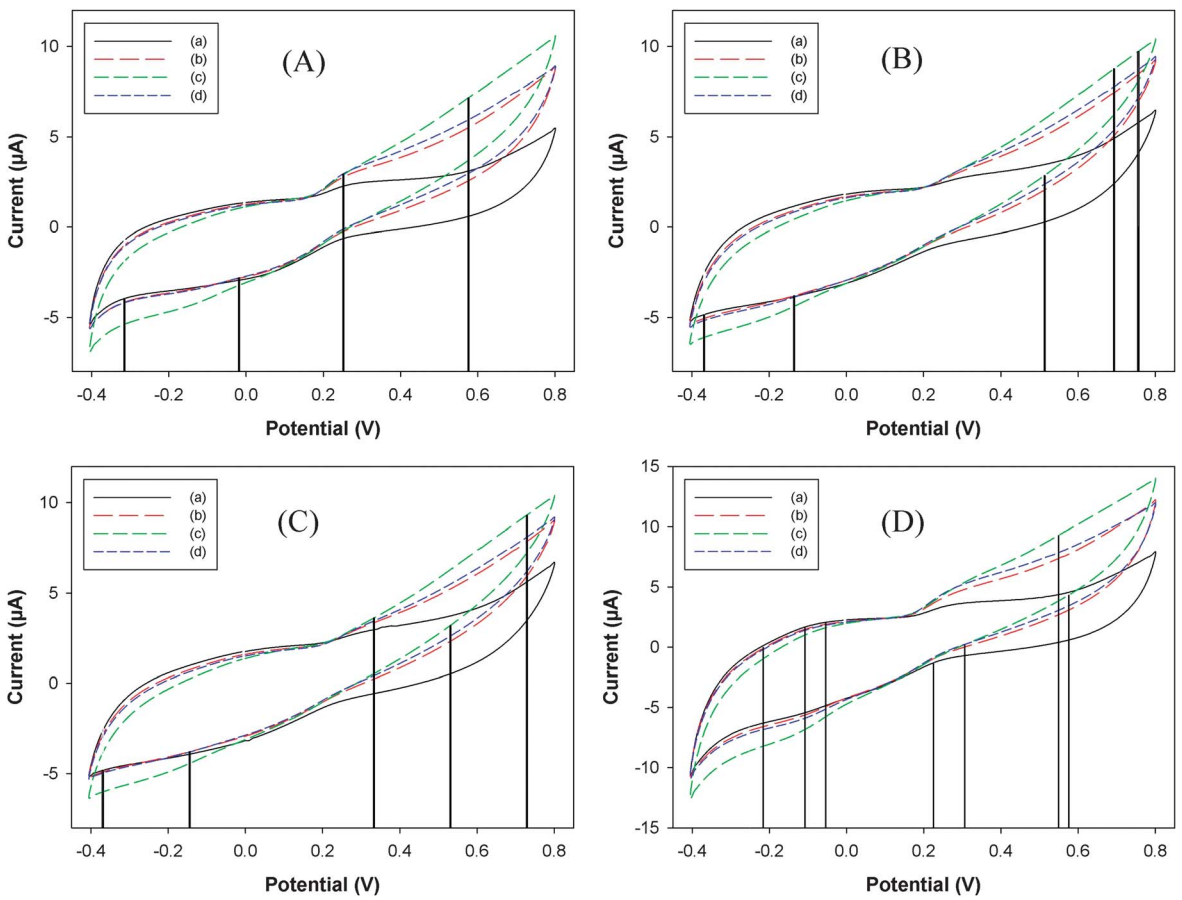

Fig. 2 Examples of the different obtained voltammograms for four mixtures of the three phenols. Concentrations for each phenol are: (a) 8.2, 39.8, 132.9 μM; (b) 107.7, 103.6, 101.9 μM; (c) 124.1, 151.7, 23.4 μM and (d) 157.7, 23.8, 86.4 μM for catechol, caffeic acid and (±)-catechin, respectively. The signals with different sensors are also shown: (A) graphite–epoxy sensor, (B) tyrosinase biosensor, (C) laccase biosensor and (D) copper nanoparticle modified sensor.

authors, and using Neural Network Toolbox (v.4.0). Sigmaplot 2000 (Systat Software Inc, California, USA) was used for graphic representations of data and results.

A total set of 37 samples, which were previously divided into two datasets, were measured: the 27 samples of the cubic design for the training set and 10 samples randomly distributed for the testing set were used. The set of voltammograms were recorded for each sample, from which some features were extracted in order to reduce the extreme complexity of these signals. The used training algorithm was the Bayesian regularization algorithm. This algorithm has the particularity that it avoids overfitting without the need to monitor the fitness degree of an internal validation subset,[51] thus this precaution is not performed.

For feature extraction selection the Back-Propagation Artificial Neural Network (BP-ANN) method was used. This method usage for variable selection is the finding of an optimal set of inputs that can successfully classify or predict the desired outputs. It is a feed-forward network and combines a back-propagation algorithm which is used to train the network according to a learning rule.[52]

An exhaustive study in the ANN architecture and configuration was done in order to optimize the separate quantification of the three phenols considered. Assessment of learning accomplished was estimated from the residual values calculated between expected and found concentration values, for each sample (i) and for each of the three analytes (j) considered:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{ij}\left(c_{ij} - \hat{c}_{ij}\right)^2}{3n - 1}} \quad (1)$$

Results and discussion

Voltammetric responses

Examples of the different obtained signals coming from the voltammetric biosensors for different phenolic compounds are shown in Fig. 2. Differentiated signals were observed for different kinds of biosensors, but due to signal overlapping it was not possible to assign a specific reduction or oxidation peak potential to each phenolic compound. Despite it being not possible to differentiate potentials, the fact is that the sensitivity towards each compound is slightly different; current intensities increase in different ways, which was confirmed when performing calibration curves of the different biosensors (as shown in the ESI, Fig. S1‡), obtaining different slopes for each compound.[23] The different relative responses of each phenolic compound arise in the different enzyme affinity towards the compound or in the electrochemical activity in the case of the copper modified biocomposite. This situation, with marked mix-response for the different involved compounds and with differentiated response from the different biosensors used, is highly desirable for studies with BioET systems.

Feature extraction

As already mentioned, it was very difficult to discriminate between individual peak signals of the three phenols in the voltammograms when the mixture was measured; when the stock solutions of each compound were treated independently it was possible to observe at which potentials there were appearing some maxima and the sensitivity differences for each one. Therefore, to carry out the dimensionality reduction and feature selection, BP-ANN was used.[53,54]

First of all, the number of neurons in the hidden layer is selected in advance to represent the intrinsic dimensionality of the dataset. This represents a limit on the number of hidden neurons in the initial ANN. Based on experience and literature, the suggested number of neurons in the hidden layer should be already very close to the optimal size before the network reduction procedures are applied, usually five neurons is a good number. Thus, most of the effort is directed at reducing the number of network inputs since the number of outputs is fixed by the requirements of the model.

Afterwards, the next step is to determine the importance of network inputs. The relative contributions of the inputs to the variance of the output layer in the trained network are calculated. Once the ANN model is trained, the analysis of its connection weights can easily identify the important inputs. Inputs that make relatively small contributions to the variance can be discarded. These small contribution values indicate that either the input does not change significantly or that the training of the ANN has allocated small connection weights to scale the input. Repeating the process of training the ANN model with the reduced input set and the selection of the most relevant inputs can proceed until a near-optimal, small, set of inputs is identified.[55] Some of the resulting minimal sets are not unique, depending on the ANN architecture used in the training.

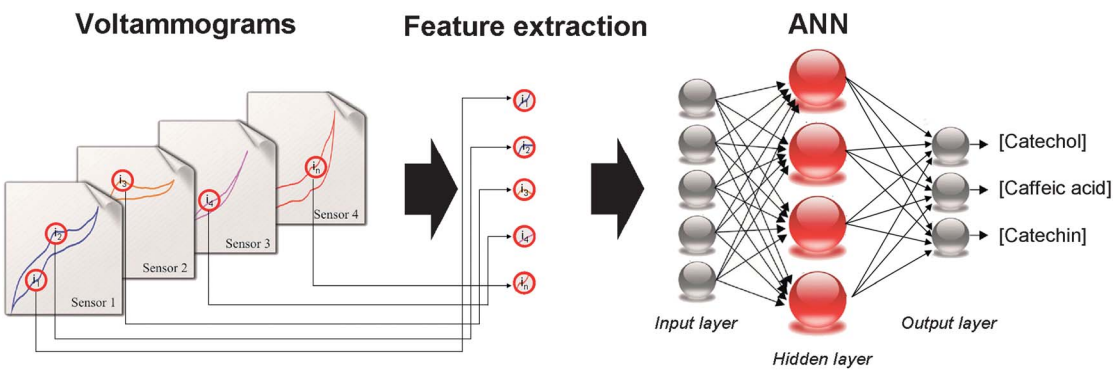

Fig. 3 Voltammetric BioET approach. Selected current intensities of the voltammograms from each biosensor are taken as the input in the ANN. Appropriate weights and biases are applied by the learning algorithm until the targets are reached within the established error.

There are advantages in identifying and removing the less-relevant inputs even if a large ANN is well trained: better modelling accuracy and higher robustness than the initial model; knowledge gain in identifying the important inputs; larger ratio of examples to ANN connection weights (thus a better generalization capacity); easier model understanding.[56] Thus, better ANN models were trained with the reduced input set, and this process was repeated until the model accuracy was degraded.

Out of the whole set of voltammograms only a number of 23 currents from the biosensor data matrix were selected, achieving an important reduction of data for the ANN input and without any loss of ANN modelling abilities. The selected potentials to which the current intensities (where "^{a}" corresponds to anodic ones and "^{c}" for cathodic) correspond for each electrode were: four currents for the graphite sensor (0.252^c, 0.576^c, −0.018^a and −0.315^a V), five for the tyrosinase biosensor (0.693^c, 0.756^c, 0.513^a, −0.135^a and −0.369^a V), five for the laccase biosensor (0.333^c, 0.729^c, 0.531^a,−0.144^a and −0.369^a V) and nine for copper nanoparticles sensor (0.549^c, 0.576^a, 0.306^a, 0.225^a, −0.054^a, −0.216^a, −0.252^a, −0.108^c and −0.054^c V).

Quantification of the three phenolic mixtures

According to the above, 23 current intensities at specific potentials for the array of sensors were selected as input vector in the ANN, the corresponding concentrations being the targets that the modelling should reach. The architecture of the ANN used was defined by these data; the input layer consisted of 23 neurons with a linear transfer function and the output layer consisted of three neurons, namely the three concentrations sought. We used a single intermediate layer, known as the hidden layer, whenever its number of neurons was specifically studied. Final data modelling was carried out using a feedforward architecture and Bayesian Regularization, as this training algorithm showed good capacity for training and prediction. Fig. 3 shows the scheme of this ANN based approach.

Once the algorithm was selected, an exhaustive search of the ANN architecture was performed. For this proposal a systematic study of the number of neurons in the hidden layer and combinations of functions in both hidden and output layers were tested. The number of neurons was changed from 1 to 10 and combinations of *tansig* (hyperbolic tangent sigmoid transfer function), *logsig* (log sigmoid transfer function), *purelin* (linear transfer function), *satlin* (saturating linear transfer function) and *satlins* (symmetric saturating linear transfer function) transfer functions were tested.

Once the modelling was completed, its RMSE value was calculated and an overall comparison was performed, as shown in Fig. S2, ESI‡. In addition, the predicted ANN concentration values were plotted against the real ones for each analyte, linear least-squares regression was fit and then slope values of the comparison and correlation coefficients were taken into account in order to help in deciding the best configuration. Therefore, the optimum model would have a small RMSE, meaning there are no differences between the values predicted by the ANN and the real ones. Also the comparison graphs should display a correlation value and slope close to 1 and intercept close to 0, giving the comparison trend between the results obtained by the BioET and the real ones and the model capacity. Each architecture was retrained five times, from a different random reinitialization of its weights, this being the source of the interval errors included for each point in the figures.

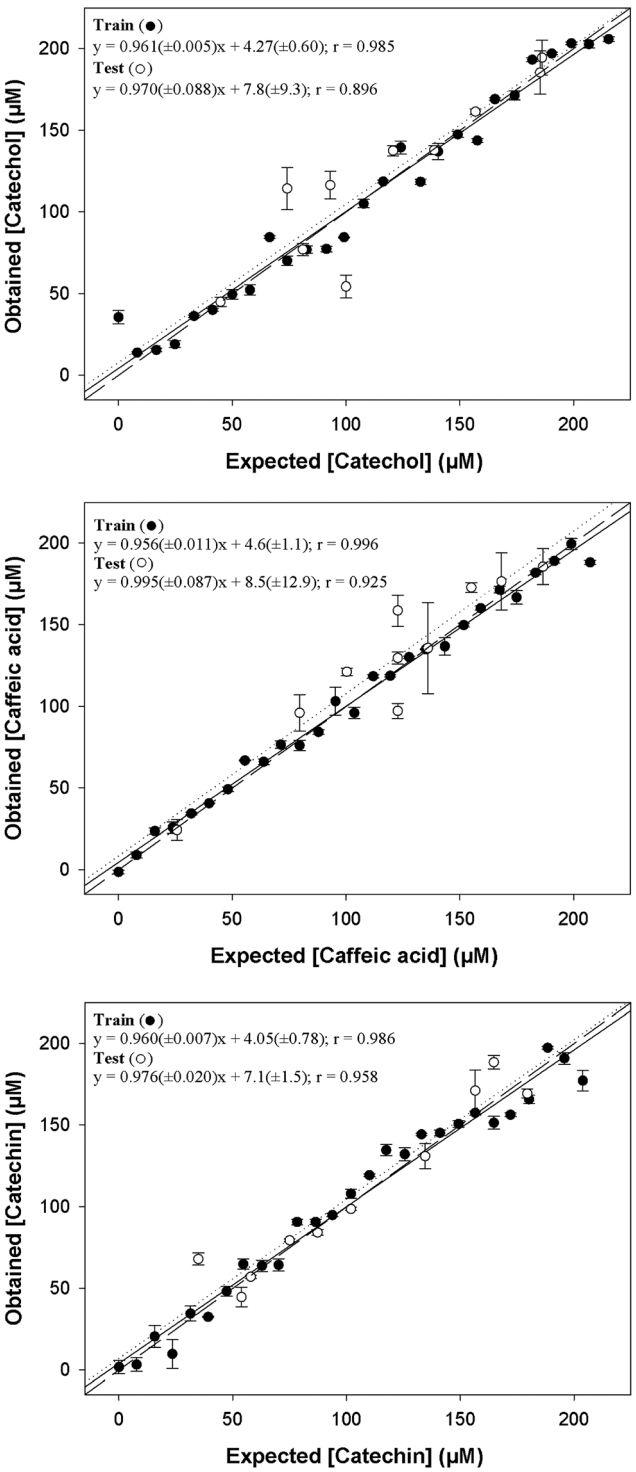

Fig. 4 Modelling ability of the optimized ANN. Training (●, solid line) and external test (○, dotted line) set adjustments of the expected concentration *vs.* obtained concentrations for (top) catechol, (middle) caffeic acid and (bottom) (±)-catechin. Dashed line corresponds to theoretical diagonal line. Error bars correspond to 5 different retrainings with random reinitialization of weights for the final architecture.

Table 1 Detailed results obtained for some synthetic samples against the real concentration. Recovery yield is also expressed for each synthetic sample

	Catechol			Caffeic acid			(±)-Catechin		
	BioET/ μM	Spiked concentration/ μM	Recovery yield (%)	BioET/ μM	Spiked concentration/ μM	Recovery yield (%)	BioET/ μM	Spiked concentration/ μM	Recovery yield (%)
Sample 1	45.02	44.81	100.5	121.27	100.31	120.9	169.31	179.41	94.4
Sample 2	76.92	81.00	95.0	24.34	25.70	94.7	79.47	75.02	105.9
Sample 3	194.42	186.12	104.5	135.73	135.96	99.8	44.65	53.82	83.0
Sample 4	54.33	99.96	54.4	97.25	122.70	79.3	57.08	57.90	98.6
Sample 5	137.59	120.64	114.1	172.78	155.03	111.4	130.89	134.56	97.3

Checking the RMSE values obtained, it was concluded that the models using the *logsig* function in the output layer yielded higher RMSE values and also they had comparison slopes lower than 0.3, therefore these configurations were discarded. When *satlin* function was used in both hidden and output layer, unsatisfactory models with also bad regression behaviour were observed, which were also discarded. The same behaviour was obtained for *satlins* function, except when it was used in the output layer combined with *tansig*, *logsig* or *purelin* functions in the hidden layer. It can also be seen that the best RMSE values were obtained with three neurons in the hidden layer with similar RMSE values for the remaining configurations, except for *purelin–purelin*, *purelin–satlins* and *purelin–tansig* which were also discarded.

For the final selection of the best ANN we also considered the comparison of modelling performance for each analyte. In this way, despite the best RMSE values being obtained with three neurons in the hidden layer, there was a high improvement in both slope and correlation coefficient when the number of neurons was increased to 5. For all these reasons, the optimum configuration chosen was the one with 23 neurons in the input layer, 5 neurons and *logsig* transfer function in the hidden layer and 3 neurons and *purelin* transfer function in the output layer.

Fig. 4 shows the training and testing performances for the three analytes with the optimal ANN configuration, where its modelling and prediction capability is demonstrated. It must be remarked that the external test subset data are not employed at all for the modelling, so its goodness of fit is a measure of the accomplished modelling performance. In both cases very good correlation was obtained, with regression lines almost indistinguishable from the theoretical ones.

Error bars plotted and stated uncertainties of fitted lines correspond to 5 different retrainings with random reinitialization of weights for the final architecture, giving information about modelling reproducibility. When statistical analysis is done on each regression separately, information more related to the data dispersion is obtained. Calculating the complete regression statistics for one of the above retraining cases, slopes were also very close to 1 and intercept close to 0, containing both values in the confidence interval, and with no significant variation in the correlation coefficient. Confidence intervals for training are one order of magnitude higher (*e.g.* catechin: $y = 0.956(\pm0.073)x + 4.5(\pm8.7)$; $r = 0.983$), and the ones for testing are even slightly greater (*e.g.* catechin: $y = 0.996(\pm0.233)x + 5.8(\pm26.9)$; $r = 0.961$). These larger uncertainty values are explained by the observed dispersion in the graphs and the lower number of points in this subset. Prediction capability of the model could be considered very good, as is summarized in Table 1, in which the recovery yield for the three polyphenols and random selected test samples is specified.

Furthermore, in order to demonstrate the abilities of the proposed BioET, some wine samples were spiked with variable amounts of the three phenolic compounds randomly distributed in the range of the experimental design. These were prepared and analyzed employing the same ET and data treatment (selected features extraction plus optimized ANN architecture). In this

Table 2 Detailed results obtained for the spiked wine samples against added concentrations of the three phenolics considered. Recovery yield is also expressed for every wine sample

	Catechol			Caffeic acid			(±)-Catechin		
	BioET/ μM	Added concentration/ μM	Recovery yield (%)	BioET/ μM	Added concentration/ μM	Recovery yield (%)	BioET/ μM	Added concentration/ μM	Recovery yield (%)
Wine 1	38.49	49.98	77.0	115.20	108.60	106.1	170.18	177.78	95.7
Wine 2	178.17	178.37	99.9	82.07	92.85	88.4	37.60	12.64	297.5
Wine 3	17.26	17.23	100.1	66.36	64.67	102.6	132.18	200.61	65.9
Wine 4	115.06	75.83	151.7	159.35	197.31	80.8	168.71	161.47	104.5
Wine 5	148.71	143.04	104.0	131.62	131.82	99.8	119.19	116.61	102.2
Wine 6	151.34	157.69	96.0	58.25	30.26	192.5	26.62	26.10	102.0
Wine 7	92.37	93.92	98.3	40.92	19.48	210.0	69.25	58.72	117.9
Wine 8	49.66	36.84	134.8	44.16	41.45	106.5	108.70	98.67	110.2
Wine 9	117.84	118.05	99.8	123.46	156.69	78.8	123.70	75.84	163.1
Wine 10	158.04	202.50	78.0	177.40	174.10	101.9	83.49	140.26	59.5

case, given that the wine matrix is quite different from the standard buffered solution which was used to build the first response model, the system was retrained to compensate for any matrix effect. Then, employing a leave-one-out validation method forced by the reduced number of samples and to ensure each sample in the set is used once for validation, the prediction capability of the new response model for wine samples was evaluated. Also in this case, the recovery yield for the three polyphenols was calculated, which is summarized in Table 2. As can be seen, the obtained results are satisfactory with a good recovery yield in general (average values of 104.0%, 116.7% and 121.9% for catechol, caffeic acid and catechin, respectively).

Compared with previous works in which departure information was obtained from a single sensor, in this work, an array of voltammetric biosensors has been used which represents a novelty in the field of ETs. To confirm that the approach was necessary, and that no redundant information was used, the ANN modelling was also attempted using the information from a single voltammogram. In this case, and with little differences depending on the sensor used, reasonable models were still obtained, only they showed a problem in the quantification of caffeic acid. In all cases, comparison slopes were lower than 0.9 (even as bad as 0.3), correlation coefficients were as low as 0.90 in general, or even 0.80 for caffeic acid; finally RMSE values were also greater (between 9 and 13 μM for the best cases, instead of 5–6 μM which are the values obtained with the biosensor array). These final details, especially when compared with the BioET approach, can serve as a contrast of its utility, and validate the presented approach. Moreover, when dealing with wine samples the gain provided by the BioET which helps avoiding all matrix effects and providing a useful tool to carry out resolution of phenolic compound mixtures is clearer.

Conclusions

In summary, a very good quantification of the three phenolic antioxidant compounds has been achieved, by using a bio-Electronic Tongue which employs the combined response from different enzyme biosensors to complement the analytical departure information and ANNs as the tool for building the response model. Resolution of signal overlapping by this chemometric tool permitted the quantification of three important phenols in the beverage field such as catechol, caffeic acid and catechin, in an application comparable to more complex analytical techniques such as HPLC. The developed BioET has been evaluated with aqueous and wine samples, with good results in both cases, demonstrating its potential as a tool for phenolic mixtures resolution in this field. With a proper retraining samples set, the system may be alternatively applied for the quantification of analogous or different phenolic mixtures in real wine samples or other beverages.

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