Chapter 7

Continuous monitoring based on biosensors coupled with artificial intelligence

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1. Introduction

Nowadays, the accurate measurement of significant parameters is an essential task for scientific and industrial fields. As examples we can mention, the monitoring of glucose for diabetic people and the environmental monitoring of emergence pollutants, such as pesticides or heavy metals, derived from industrial activity (Ogrodzki 2009, Mimendia, Gutierrez, Leija, Hernandez, Favari, Munoz et al., 2010). The evaluation of toxic compounds in food and beverage products before human consumption is an essential task as well. To carry out monitoring, specialized instrumentation such as mass spectrometry, gas or liquid chromatography and electrophoresis techniques are applied. The listed methods usually offer reliable results, ensuring that that the desired parameter is measured with high sensitivity, selectivity and accuracy even in complex samples (e.g. food samples, human blood or polluted river water) where interference can devalue the instrument response (Rodriguez-Mozaz, Lopez de Alda & Barcelo, 2007). However, a current trend in monitoring is the on-line, continuous acquisition of data from parameters of interest. Most of the mentioned methods are designed for a controlled laboratory environment rather than for an *in-situ* and continuous measurement.

As an alternative to laboratory techniques, chemical sensors were proposed as simple analytical tools providing selective information about a specific analyte in the sample. Biosensors are a special group of chemical sensors, where a biological material is used as bioreceptor for getting highly specific information about a specific analyte in the sample (Thevenot, Toth, Durst & Wilson, 1999). Nowadays, biosensors development is a growing field with thousand of publications every year devoted to environmental, food and beverage industry, security and medical applications (Ivnitski, Abdel-Hamid, Atanasov & Wilkins, 1999; Rodriguez-Mozaz, Marco, de Alda & Barcelo, 2004; Campas, Prieto-Simon & Marty, 2007; Arduini, Amine, Moscone & Palleschi, 2010). Since biosensors are base on molecular recognition, devices for specific compounds can be potentially designed for fast, low cost and portable applications such as the hand held glucose meter for diabetic people.

In spite of their widespread in scientific literature, very few biosensors have reached commercial success outside of laboratory tests. One reason could be, the single analyte approach, which is a disadvantage when compared with some powerful multianalyte established techniques (Luong, Male & Glennon, 2008). To enhance biosensor performance chemometric tools have been proposed as sensitive calibration models. Especially artificial neural networks (ANN) have been proposed as calibration tools because their ability to model non linear signals, commonly found in biosensors (Almeida, 2002). In addition, a multianalyte approach to provide information about several analytes present in sample is possible with ANN modeling (Bachmann, Leca, Vilatte, Marty, Fournier & Schmid, 2000). In spite of their advantages, chemometric tools have been barely applied to biosensors but when applied they have shown an improvement in the whole system performance.

This chapter is devoted to the review of artificial neural networks in the field of biosensors. Papers from 1995 to 2012 are presented and their applications for modeling, calibration tool for multianalyte approach is presented. Biosensing platforms applied to on-line detections in industrial process or environmental monitoring are also introduced.

2. Biosensors

2.1. Principles

In the broad sense, a sensor is a device able to convert the measurand into a measuring signal. For chemical sensors, the measurand is usually a chemical property or a specific component (analyte) in a sample of interest. The sensing information may be originated from a chemical reaction of the analyte or from the change of a physical property in the sample (Thevenot et al., 1999).

Chemical sensors have two basic sequentially connected components, namely receptor and transducer. When the receptor is based on a biochemical mechanism the whole device is called a biosensor, which is shown in figure 1. According with the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is an integrated receptor-transducer device which is capable of providing selective quantitative or semi-quantitative analytical information about a specific analyte using a biological recognition element (Thevenot, Toth, Durst & Wilson, 2001). In the beginning, biosensors main applications were intended for the biomedical field (i.e. monitoring of biological samples such as glucose in human blood), but the current trends in biosensing monitoring include environmental, food, pharmaceutical and security fields (Luong et al., 2008).

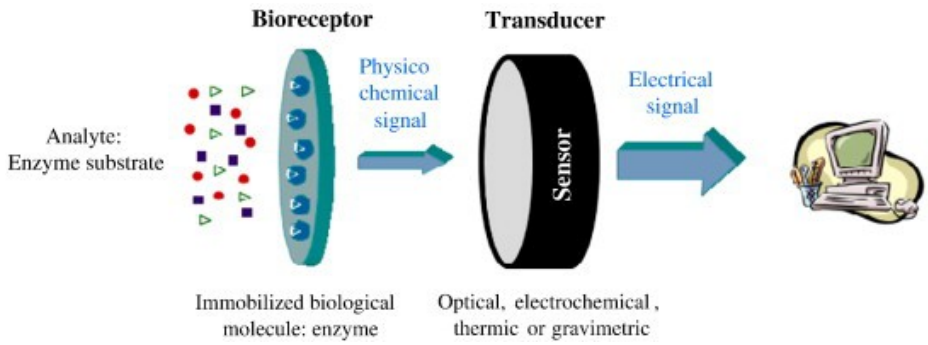

Figure 1. Typical representation of a biosensor

Biosensors can be classified according with their biological recognition method or with their signal transduction mode. For the biological recognition method two main categories are distinguished: biocatalytic devices and affinity sensors (Ronkainen, Halsall & Heineman, 2010). In biocatalytic sensors enzymes, whole cells (i.e. bacteria, fungi, eukaryotic cells) and slices of tissues (plants or animals) are used as recognition element. In these devices, the immobilized bioreceptor catalyzes a reaction which produces a detectable compound. For this group, enzymes are the most commonly found bioreceptors. The advantages of biocatalytic biosensors are the compact, easy to use and cheap design, which is exemplified in the commercially successful blood glucose biosensor (Luong et al., 2008; Chen, Xie, Yang, Xiao, Fu, Tan, et al., 2013). However, when analytes of interest are not noticeable by biocatalytic receptors or their inherent selectivity is affected by components present in complex samples, affinity biosensors are preferable. In this group the final signal is the result of the highly selective binding between

the target analyte and a biomolecule (e.g. antibody). The recognition is determined by the complementary size and shape of the binding site to the analyte of interest.

In addition to bioreceptor, biosensors can be classified accordingly with their transducer mode. Electrochemical transducers cover the majority of the current reported literature on biosensors, while optical, piezoelectric and thermal transducers represent the minority (Thevenot et al., 2001). For the electrochemical transducer, the interaction between the bioreceptor and the target analyte produces detectable electroactive specie that can be measured as a current (amperometric detection) or a potential (potentiometric detection). Changes such as conductive properties in the medium or alteration on the resistance can be electrochemically detected as well. Transducers based on optical methods can transform a change in the reflectance of the surface, which is induced as a result of the interaction between the bioreceptor and the analyte. For this group, surface plasmon resonance and spectroscopy are among the most commonly found techniques in literature and in commercial available systems.

2.2. Single analyte detection

Regardless the classification, the highly selective detection of a target analyte is one of the main (if not the main) features of biosensors. In all the cases, as a result of this selective interaction a well defined signal proportional to the concentration of the target analyte is expected. For quantitative information, a mathematical relationship is established between stock solutions with well-know concentration of the studied analyte and the resulting signal. Usually, a specific representative feature of the signal (e.g. maximum current, maximum absorbance) is chosen to be related with the known concentration trough a simple linear model. Biosensor performance is tested with new samples with unknown concentration; the accuracy of determination is analytically evaluated by the coefficient of determination (R^2) and the recovery percentage. This procedure is plenty in specialized literature and suitable for controlled laboratory conditions for single target analyte detection.

2.3. Biosensor arrays

A single test is intrinsically implicit in biosensor operation since a single device can provide information about only one specific component of the analyzed sample. This can be a restriction for biosensor inclusion in analytic applications where a multianalyte approach is preferred (i.e. medical diagnosis, environmental monitoring) (Mimendia, Legin, Merkoci & del Valle, 2009; Escuder-Gilabert & Peris, 2010; Mimendia et al., 2010). For this issue sensor arrays are a good alternative. The concept of sensor array was firstly applied for gaseous sample in the analytic devices known as electronic noses (EN). Later, the concept was extended to liquid samples in the electronic tongue (ET) device. Both, EN and ET were able to provide analytical information of complex samples, while keeping a simple and relative low cost instrumentation. The main features were the inclusion of sensors with different selectivities and sensitivities towards multiple analytes and the use of high order chemometric tools for data processing (del Valle, 2010; Escuder-Gilabert & Peris, 2010). The extension of this methodology to biosensor field was possible with the inclusion of new technologies for the development of micro arrays with different immobilized bioreceptors and the inclusion of instrumentation for multichannel measurements. Besides, new immobilization methods for bioreceptors, the improving of existing biosensors and the inclusion of novel biosensors for new analytes of interest will result in wider analytical applications for biosensor arrays.

3. The role of Artificial Neural Networks in biosensor applications

3.1. Foundations of Artificial Neural Networks in biosensor data modeling

The main interest for a chemical model is to establish a relationship between a set of measurements and a set of target results (e.g. concentrations) (Rodionova & Pomerantsev, 2006). This mathematical relationship has been modeled by several ways, but in the last decades chemical field underwent a revolution with the development of sophisticated complex equipment along with high speed computer facilities, resulting in large amount of data (chemical data). Therefore, there was an urgent need to analyze such as higher order information with propel models to extract meaningful information. This fact promoted the development of the chemistry discipline known as chemometrics, which basically is dedicated to find relevant information from the measured chemical data (Rodionova & Pomerantsev, 2006). Several models (linear and non-linear) are developed, covered and studied by chemometrics. Within these models, ANNs are of great interest as modeling and calibration tools (Marini, 2009). ANNs are mathematical models attempting to mimic biological neural networks functioning in a simplified way. Even historically ANNs are related with Artificial Intelligence, they have been successfully applied in several branches of analytical chemistry (Smits, Melssen, Buydens & Kateman, 1994). History of Artificial Intelligence as discipline and ANN models has been covered by several reviews and book chapters and will not be covered here extensively. The main focus of this section is the application of ANN models for chemical science, in particularly for biosensors and biosensor arrays as alternative methods to obtain relevant information for analytical purposes.

The main ANN architectures found in chemical literature are Multilayer Perceptron (MLP) and Radial Basis Function (RBF). For MLP, usually a three-layer architecture is preferred but architectures with more layers can be found as well. When a single biosensor is modeled the input number of variables is defined by the meaningful features of biosensor measurement (e.g. peak height, peak area, maximum absorbance or intensities). When a biosensor array is modeled the number of neurons in the input layer is the number of elements in the array. Components in the output layer are usually the expected values for target analytes, but prediction of biosensor behavior can be also found as ANN output. A three layer MLP can be described as the computation of N_o functions of N_i input variables. Each function is a weighted combination of the non-linear functions computed by the neurons in the hidden layer that can be expressed as follow:

$$y_k(x) = g\left(\sum_{j=1}^{N_h} w_{kj} f\left(\sum_{i=1}^{N_I} w_{ji} x_i + w_{j0}\right) + w_{k0}\right)$$

Where

y_k is the kth component of the output vector

w_{kj} hidden to output weight

w_{ij} input to hidden weight

f and g are the non-linear activation functions

Fixing the weights in ANN architecture to find the values that best map the input experimental data to the desired output (i.e. optimization) is generally performed by the back propagation training algorithm. The goal is to change iteratively the weights between neurons in a direction that minimizes the error (E), according to the steepest descendent method (Marini, 2009).

A different architecture barely found in biosensor modeling is the Radial Basis function neural network (RBF-NN). While architectures of RBF-NN and MLP are similar, the differences are in the activation function used by RBF-NN. Radial basis function, and especially Gaussian basis function, is used in the hidden layer. Other parameters to optimize in these architectures are the center, and the scale. An output for this architecture can be written as

$$y_k(x) = \sum_{j=1}^{N_h} w_{kj}\,\rho(\|x-\mu_j\|)$$

Where

y_k is the kth component of the output vector

w_{kj} hidden to output weight

ρ radial basis function

x input vector

Preprocessing of biosensor signals is usually focused on extracting meaningful features to feed the ANN model (Jakubowska 2011). However and since biosensors are designed to be highly selective to target analyte most of the signals are univariate (i.e. steady state potential, enzyme activity). However when techniques such as cyclic voltammetry are used, preprocessing stages is necessary because of the high information order (e.g. number of records) of data (Ceto, Cespedes & del Valle, 2012). This is the case for spectroscopy records, differential pulse voltammetry and transient potentiometric records. Table 1 list the preprocessing techniques found for this review along with the input signal and target analyte.

Analyte	Signal	Preprocessing technique	Reference
Polyphenols	BA[1]	Fast Fourier Transform (FFT)	(Ceto et al., 2012)
Polyphenols	BA	Windowed sliced integral method	(Ceto, Cespedes & del Valle, 2013)
Glucose	BA	Baseline correction	(Gutes, Ibanez, del Valle & Cespedes, 2006)
Glucose, urea	SB[2]	Mean values of FIAgram segments	(Hitzmann, Ritzka, Ulber, Scheper & Schugerl, 1997)
Simulation	SB	Canonical Correlation Analysis (CCA), Principal Component Analysis (PCA)	(Baronas, Ivanauskas, Maslovskis & Vaitkus, 2004)

[1]Biosensor, [2]Single biosensor

Table 1. Preprocessing techniques for chemical data

For training the model an appropriate set of well known samples is selected from the concentration range to be modeled (Smits et al., 1994). A separate set of chemical data is usually prepared for testing the trained model. The testing set is comprised by new samples, which did not account in the training and/or by real samples (e.g. juice, wine, human blood, urine). Cross

validation is the commonly applied method but some others such as jack-knife has been also reported (Marini, 2009). The accuracy of the model is measured as a function of some analytical figures of merit. The most commonly found are the sum of square errors (SSE), the root mean square error (RMSE), the relative absolute error (RAE), the coefficient of correlation (R), the coefficient of determination (R^2), the slope and intercept of the straight line formed by predicted values of ANN model *versus* the expected ones and recovery yield (Pravdova, Pravda& Guilbault, 2002; Esteban, Arino & Diaz-Cruz, 2006; Rodionova & Pomerantsev, 2006; Marini, 2009).

Finally, one of the reasons of chemometric growing is the availability of proper software for data analysis. For ANN development the table 2 list the available software and some custom implementations found in this review.

Architecture	Software	Reference
MLP	MATLAB	(Alonso, Istamboulie, Noguer, Marty & Munoz, 2012)
MLP	NEMO1.15.02	(Bachmann et al., 2000)
MLP	Turbo Pascal	(Hitzmann et al., 1997)
MLP	Stuttgart Neural Network Simulator	(Reder, Dieterle, Jansen, Alcock & Gauglitz, 2003)
MLP	Python	(Glezakos, Moschopoulou, Tsiligiridis, Kintzios & Yialouris, 2010)

Table 2. Available software for Artificial Neural Network modeling

4. Applications

This section covers the published works from 1994 to 2013 dealing with single biosensors or biosensor arrays coupled with artificial neural networks (ANN) for analytical purposes. In general ANNs are preferred as chemometric tools because their ability to accurately model non-linear data commonly found in single biosensors and biosensor arrays. The applicability of biosensors coupled with ANN is shown by the broad range fields included in this analytical methodology. Even biosensors and biosensors arrays have been modeled with other calibration tools, only those applications modeled with ANN will be considered for this section.

4.1. Modeling and simulation of biosensor response

In general, biosensors are characterized by non linear responses towards target analytes. As stated, simple calibrations do not represent accurately biosensor response in the whole range of quantification. The works in this section focus on the improvement of biosensor analytical performance towards ANN modeling. A second approach considered is the modeling of biosensor behavior after operational conditions changes. This is mainly achieved by simulation performed by ANN models and could be applied for predicting biosensor response under changing conditions

Estimation of formate with a polypyrrole based biosensor signals were performed by Talaie *et al.* (Talaie, Boger, Romagnoli, Adeloju & Yuan, 1996). Problems in the quantification were related to

uncontrolled changes in the measured signals for different formate concentrations. A preprocessing was applied in order to reduce signal drifts. Baseline correction, centering and standardization were applied to the training data set. For the centered data, the highest value in electrical current and the surrounding 21 values were taken as input values for ANN model. Conjugated gradient and sigmoid transfer functions were chosen for the final model which was not validated with an external test set. In a different study, ANNs were used as multivariate calibration tools for a single glucose oxidase polypyrrole biosensor. Calibration in the direct and inverse modeling was performed to assess both, sample concentration and biosensor performance according with the measured parameters. For this fitting function task ANN obtained correlation coefficients of 0.99 for both models (direct and inverse) with an external test set (Seker & Becerik, 2004).

Hitzman *et al*. (1997) quantified glucose and urea separately from two En-FET using glucose oxidase and urease as bioreceptor. Segments of FIAgram at a fixed analyte concentration after applying different pHs were used as ANN inputs. Prediction of glucose and urea showed an average error of 4.5% and 5.5% respectively. Quantification of phenolic content was assessed from an optical tyrosinase biosensor and artificial networks. Five absorbencies intensities measured at selected wavelengths (440,490,560 and 610 nm) were the inputs of an ANN with 23 neurons in the hidden layer and 1 neuron in the output layer, corresponding to phenol concentration. Network parameters such as number of neurons in the hidden layers and training parameters such as learning rate and epochs were extensively studied and optimized to 21, 0.001 and 30000 respectively. ANN testing with additional 10 absorption spectra data showed an improvement in the range of quantification for the studied biosensor. For simple calibration linear range was limited to 0.5-6 mgL^{-1} while ANN allowed a wider quantification range of 0.5-20 mgL^{-1} (Abdullah, Ahmad, Heng, Karuppiah & Sidek, 2008).

Mixtures of ethanol and glucose were quantified in a study exploiting non-specificity of microbial biosensors (Lobanov, Borisov, Gordon, Greene, Leathers & Reshetilov, 2001). Bacterial cells of *Gluconobacter oxydans* and yeast cells of *Pischia methanolica* were used to construct amperometric biosensors. The first bioreceptor showed a high sensitivity to glucose an ethanol, while the second showed sensitivity only to ethanol. After signal preprocessing (smoothing, removal of signal peak outburst and zero drift), the rate of change of electrode current towards binary mixtures was used as biosensor response. Obtained data for binary mixtures was represented in a three dimensional space with concentrations of ethanol and glucose as abscise and ordinate and biosensor response as applicate. In a numerical approach, the resulting surfaces for both biosensors were approximated by second order polynomials. When a new (unknown concentration) sample was used, the actual concentration was determined according with the fitting in calibration surfaces for both biosensors. Alternatively, the rates of change of both biosensors along with the time from the start of measurement were used as input for an ANN model. Normalized values were used for estimation of both glucose and ethanol with a single model. Resilient back propagation was used as training algorithm and sum of squares errors (SSE) was used to assess the accuracy of analyte determination. Final coefficients of determination for polynomial approximation were 0.976 and 0.993 for glucose and ethanol respectively, while ANN showed a R^2 of 0.995 and 0.992 for the same analytes. While polynomial approximation showed a comparable performance with ANN a wider range of concentrations can be analyzed with ANN.

In a different approach, experimental signals coming from glucose and sucrose biosensors were described with ANN models (Ferreira, De Souza & Folly, 2001). The final goal was to evaluate, by

simulation, the biosensor performance in a control closed- loop for an alcohol fermentation reactor. Inside the control loop biosensor response was simulated with a second order transfer function. Prediction between the control model and the biosensor response modeled by ANN were compared to study the possibility of including a biosensor in on-line control for alcohol fermentation process. Similarly in a simulation approach, synthesized data corresponding to mixtures were analyzed (Baronas et al., 2004). Data sets in batch and in flow mode were generated by numerical simulations following a full factorial design $M^K = 8^4 = 4096$ responses (M = substrate concentration, K = enzymatic rate) randomly divided in training and data set. Correlation coefficient analysis (CCA) and Principal Component Analysis (PCA) were applied to extract meaningful features from simulated data. CCA holds the point with the higher correlation for every input vector of data, while PCA retains statistically independent information from the same vector. ANN was used to distinguish between mixture components and to quantify their concentration. Recovery rates of 99% in both, batch and flow mode, were obtained.

Garcia *et al*. studied the interaction between substrate concentration, pH, and temperature in the final response of an acetylcholinesterase biosensor (Garcia, Burtseva, Stoytcheva & Gonzalez, 2011). A multilayer perceptron with 5 layers and 30 neurons in each one of the hidden layers was constructed to predict the behavior of biosensor response after a know variation of the input parameters. Experimental data produced after variations of substrate concentration (from 0.2 $\mu molL^{-1}$ to 1 $\mu molL^{-1}$), pH (from 5 to 9) and temperature from (25°C to 70°C) were used for training and testing network with a 5 k-fold cross validation. In addition to ANN, support vector machines (SVM) were used for modeling the same data. Average MSE% for ANN prediction was found to be 2.45% in contrast to SVM with a low average value of 0.143%. Similarly, to predict the dynamic response of a potentiometric urea flow-through De Gracia *et al*. applied ANN to a single biosensor modeling (deGracia, Poch, Martorell,& Alegret, 1996) x. A four layer network with six input parameters (height at t_1 and t_2, time, injection volume, flow and concentration) and 8 neurons in the two hidden layers was used to predict peak height after changes of one of the input values. ANN prediction was compared with experimental records as well as with deterministic model performance. While deterministic model accurately described biosensor response, ANN exhibited good prediction ability after training with a reduced data set.

4.2. Environmental monitoring

Pesticide quantification is one of the most active research areas in biosensor field. Several reviews covered the trends, advances and limitations in biosensors devoted to pesticide quantification in single and multianalyte approach (Llorent-Martinez, Ortega-Barrales, Fernandez-de Cordova & Ruiz-Medina, 2011; Van Dyk & Pletschke, 2011; Pundir & Chauhan, 2012; Liu, Zheng & Li, 2013). For pesticide quantification, inhibition of acetylcholinesterase (AChE) has been used as analytical parameter in single pesticide determinations; but for pesticide mixtures, sensor arrays with engineered AChE and multivariate calibration with ANN have been proposed and successfully applied. In the first work of his kind, Bachman *et al*. used the remaining activity of AChE multisensor array (biosensor array) after pesticide exposure to quantify the presence of malaoxon and paraoxon in binary solutions (Bachmann et al., 2000). Two arrays including wild type AChE and mutant varieties (Y408F, F368L and F368H) were constructed to generate a distinctive signal pattern for sensitive multianalyte determination (Multisensor I and Multisensor II). Network architecture was optimized using magnitude based pruning and skeletonisation methods. Models with the lower RMSE were selected for ten additional runs of 3000 epochs. Multisensor II with optimized network architecture (4 input

neurons, 5 neurons in the hidden layer and 1 output neuron) was applied for paraoxon quantification and network architecture of 4 input neurons, 4 neurons in the hidden layer and 1 output neuron was used for malaoxon determination in the range of 0-5 μgL^{-1}. Even both compounds exhibit similar inhibition behavior, after cross validation error prediction for paraoxon was 1.6μgL^{-1} and 0.9μgL^{-1} for malaoxon, showing the feasibility of this approach for quantification of compounds with similar behavior. A similar methodology was used by Cortina del Valle and Marty (2008) to quantify binary mixtures of dichlorvos and carbofuran mixtures Three AChEs (wild type AChE and engineered B1 and B394 varieties) were used as bioreceptors for the biosensor array. Enzyme activity after pesticide mixture exposure was spectrophotometrically studied by following Ellman method. For this assay, responses at wavelength 412 were used as ANN input, while for the electrochemical assay remaining biosensor activities after pesticide incubation were used to train the network. In both cases, data was normalized between -1 and 1 values. For each method, three layers ANNs were trained using bayesian regularization, values for learning rate and momentum were of 0.1 and 0.4 respectively. For spectrophotometric measurements the final ANN architecture had four neurons in the hidden layer with logsig function; amperometric measurements were modeled with 3 neurons in the hidden layer with tribas function. Optical method results showed a correlation coefficient of 0.995 for dichlorvos and 0.936 for carbofuran. By the other hand, electrochemical method showed correlation coefficient of0.969 for dichlorvos and 0.918 for carbofuran. Real samples evaluation for both methods le to recovery rates range of 89-116%. Following the same strategy, binary mixtures of chlorpyrifos oxon (CPO) and chlorfenvinfos (CFV) were evaluated with two different three layers ANN (Istamboulie, Cortina-Puig, Marty & Noguer, 2009). Both models had four neurons in the hidden layers, but tansig transfer function was used in the first one (ANN_1) and logsig transfer function was used in the second one (ANN_2). Correlations of 0.998 for CPO prediction and 0.995 for CFV were obtained with the first model. Correlations of 0.997 for CPO prediction and 0.986 for CFV were obtained with the second model. Spiked samples were prepared to probe the accuracy of ANN modeling resulting in recovery rates of 98% for the tested concentrations. One of the main drawbacks of methodologies based on measuring the remaining enzymatic activity after incubation of pesticide is the prolonged analysis time. Thus, an alternative method based on the slope of inhibition caused by the immediate interaction of pesticide over enzyme activity in steady state (without incubation time) was used by Alonso *et al*. for pesticide mixtures quantification (Alonso et al., 2012). In this study three electrodes based on AChE (B131 and B394 varieties) were exposed to mixtures of CPO, CFV and azinphos-methyl oxon (AZMO). The slope caused by pesticide inhibition was used to train a three layer ANN model with 3 neurons in the input layer, 95 neurons in the hidden layer and three neurons in the output layer. Values for training algorithm were studied in the range of 0.1 to 0.3 for learning rate and 0.4 to 0.9 for momentum. Network performance was evaluated according to the lower RAE. Final model resulted in a RAE of 1.82% for CPO (r = 0.985), 1.51% for CFV (r = 0.991) and 2.3% for AZMO (r = 0.997). Real samples were applied to the model for simultaneous determination of pesticide concentration. For the evaluated concentrations recovery rates were in the range of 92.05 to 105.31% for 20 evaluated samples. A method based on the spectrophotometric measurements of enzymatic kinetics was used for determination of mixtures of carbaryl and phoxim Ni *et al*. (Ni, Deng, Kokot, 2009). The signals were processed with Radial Base Function Neural Network (RBF-ANN). In addition, the highly non linear behavior of enzymatic kinetic data was processed with chemometric linear methods such as PLS2, PLS1 and PCR. The efficiency of proposed models was evaluated according to the relative prediction errors (RPE). For the linear methods RPE values

were in the ranges between 8.3-15.5% for both pesticides, while for RBF-ANN model RPE value resulted in 5.2% for carbaryl and 6.5% for phoxim. The accuracy of RBF-ANN model was probed in spiked samples of lake water. Satisfactory recovery rates were obtained in the range of 98.8-103% for carbaryl and phoxim using this model.

Until now, all listed applications used biocatalytic biosensors taking advantage of the selectivity showed by AChE family towards pesticide compounds. The work proposed by Reder *et al*. employed the cross reactivity of two polyclonal antibodies to quantify the presence of two analytes: atrazine and simazine (Reder et al., 2003). Calibration experiments, dealing with the optimization of antibody presence (mixed or separated), were performed according with a full factorial design. Obtained signals were mean centered and autoscaled before ANN training. While in all cases prediction performance was lower than expected, the inclusion of different antibodies could improve the discrimination of triazines.

In a different application intended for environmental monitoring, Gutes *et al*. (2005) proposed the multivariate calibration of polyphenol oxidase amperometric biosensor with ANN for the quantification of three polyphenol compounds namely phenol, catechol and m-cresol. Synthetic mixtures of three polyphenols were electrochemically analyzed with linear sweep voltammetry. The record containing 34 measurements intensities was used to feed ANN without any preprocessing. For the ANN architecture Bayesian regularization was used as training algorithm and neuron number in the hidden layer was investigated along with a combination of transfer functions with the hidden an output layer. A combination of logsig and purelin, with five neurons in the hidden layers was chosen as optimal architecture according with the low RMSE obtained as compared with different combinations. Network predictions were near to optimal values with correlation coefficients of 0.988 for phenol, 0.997 for catechol and 0.993 for m-cresol. Even the final goal of the work was to apply the develop methodology to environmental pollution real samples analysis was not performed.

Contamination by mycrocystins mixtures (LR and YR) was assessment by modeling inhibitions of two protein phosphatase biosensors with ANN (Covaci, Sassolas, Alonso, Munoz, Radu, Bucur et al., 2012). Signals from different incubation times (20, 30 and 60 minutes) were analyzed. The network was trained using resilient back propagation, learning rate of 0.05, momentum of 0.005 and logsig transfer function. The final architecture for the network was 2 input neurons, 10 neurons in the hidden layer and two neurons in the output layer. Signals from 30 minutes of incubation time were chosen for the final model. The obtained correlation for LR was 0.996 and 0.983 for YR, while absolute errors in the range of 0,0012 to 0,0073 nM for LR and 0,012 to 0,055 nM for YR. For testing subset average recovery yields for LR was 101.73 and 105.66 for YR.

4.3. Food analysis

Besides the environmental pollution, polyphenols are present in a variety of classes as natural antioxidants. This is the case of wine and beer where polyphenols are responsible of antioxidant properties and organoleptic properties (e.g. bitterness, astringency and harshness) (Rodriguez-Mozaz et al., 2004; Ceto et al., 2012). Methods to quantify polyphenols include the Folin-Ciocalteu (FC) method, the polyphenol index (I_{280}) and High Performance Liquid Chromatography (HPLC). An alternative methodology based on a bioelectronic tongue to quantify the total polyphenol content in wine samples was proposed by Cetó *et al.* (Ceto et al., 2012). The biosensor array was comprised by four voltammetric sensors with tyrosinase or laccase enzymes as bioreceptors. The studied polyphenols were gallic acid, (±)-catechin, p-coumaric acid, caffeic

acid, catechol, phenol, m-cresol, ferulic acid, chlorogenic acid and quercetin. For a first qualitative analysis, 200 µM of each of the listed polyphenols were added to different wine samples with low polyphenol content. The enhanced individual polyphenol samples were analyzed with the biosensor array and the obtained signals preprocessed using PCA. The three first components were used to represent data, with an accumulated variance of 99%. Eleven distinguished classes (10 individual polyphenols, plus wine samples without extra added polyphenol content) were classified with and ANN fed with the PCA scores. The network with 3 neurons in the input layer, 7 neurons in the hidden layer and 11 neurons with logsig function in the output layer classified the whole data. Thus sensitivity and specificity of the ANN classifier was 100% for both parameters. For a quantitative approach an ANN model was trained to predict total phenolic concentration from biosensor response after analysis of 29 wine samples of different varieties. Original biosensor array information with dimension 268 x 4 x 29 (intensities recorded x number of sensors x number of wines) was pre-processed with Fast Fourier Transform (FFT) before network training. The first 32 Fourier coefficients were used to represent each signal, leading to a compression of 88.1% as compared with the original information. Network architecture for quantification was fixed in 128 input neurons (32 coefficients for each sensor signal), 6 neurons in the hidden layers with logsig function and two output neurons with tansig function. The results provided by the ANN model were compared with established methods FC and I_{280}.The ANN methodology obtained a correlation coefficient of 0.978 when compared with FC method and of 0.949 when compared with I_{280}. The main advantage showed by this monitoring proposal is the simplicity, low cost, sensitivity and rapidity. The same group, performed and individual polyphenol analysis in beer samples following a similar methodology (Ceto et al., 2013). Basically, the same biosensor array was used to analyze synthetic mixtures of ferulic acid, gallic acid and sinapic acid. Resulting signals were pre-processed using windowed slicing integral (WSI) method. WSI divided the signals into k sections; for feature extraction the area under each section is calculated and taken as a representation (coefficient) of that specific segment of signal. For this application, biosensors signals were divided in 11 sections, and therefore represented by 11 coefficients. A network architecture of 44 input neurons, 5 neurons in the hidden layer (with tansig transfer function) and 3 neurons in the output layer provided, respectively, correlation coefficients of 0.977, 0.988 and 0.978 for ferulic acid, gallic acid and sinapic acid for testing set. Spiked real samples were applied to the system resulting in average recovery yields of 103%, 103% and 106% for ferulic, gallic and sinapic acid.

4.4. Biomedical applications

A quantification of urea and interfering alkaline ions (ammonium, potassium and sodium) with a potentiometric bioelectronic tongue in urine samples was carried out by Gutierrez et al. The biosensor array comprised all-solid state potentiometric chemosensors and biosensors modified with urease enzyme. The approach was proposed as a simple method for simultaneous quantification of urea and their common interference species in clinical samples. The data set produced by biosensor array was designed accordingly to a fractional factorial design with three levels and four factors (3^{4-1}). Calibration was performed by linear and non-linear tools (PLS1 and ANN). Network architecture of 12 input neurons (corresponding to 12 sensors in the array), 5 neurons in the hidden layers with tansig function and 4 neurons in the output layer was chosen as optimal. The network was trained with Bayesian regularization algorithm, learning rate of 0.1 and momentum of 0.4. Network architecture showed a RMSE = 0.0024 when applied to 10 additional samples used as testing set. Correlations of 0.81, 0.978, 0.995 and 0.992 were

obtained for urea, ammonium, potassium and sodium respectively. The PLS1 model was tested with the same additional samples and obtained correlations of 0.727, 0.818, 0.994 and 0.919 for the same analytes. The superior performance of ANN could be attributed to the highly non-linear nature of biosensor array signals, which was accurately modeled by the non linear transfer functions in the hidden layer of ANN model. Finally, 18 urine samples were presented to both models to assess their accuracy in real clinical applications. Urine samples were previously analyzed with reference determination procedures and the obtained results compared with those computed by the models. Both, ANN and PLS1 showed a lower performance as compared with synthetic samples with slopes and intercepts away from ideal values when compared with reference methods. For ANN model this could be attributed to the high variability introduced by urine matrix in the biosensor array response, which is not considered in the training data set. Final average errors were 8% for ANN and 13% for PLS1.

The same authors extended this study adding creatinine as fifth analyte. By following the same methodology, a biosensor based on creatinine deaminase was added to the biosensor array (Gutierrez, Alegret & del Valle, 2008). Training parameters were kept but neurons in the hidden layer were fixed to 6. A superior performance as compared with the previous study was observed in the real urine samples testing. Achieved correlation coefficients were 0.967, 0.94, 0.97, 0.995 and 0.991 for urea, creatinine, ammonium, potassium and sodium respectively. However slopes and intercepts were near to ideal values when compared with reference established methods. This could be attributed to the optimization of biosensor array as compared with the first study, showing the importance of significant and meaningful input signals for an accurate modeling.

In this section some important analytes in clinical analysis such as glucose and urea were determined by biosensor array and ANN modeling. These studies probed the potential application of a simple and low cost methodology with reasonable performance for clinical samples analysis.

4.5. Agricultural applications

Identification of *Tobacco Rattle Virus* and *Cucumber Green Mottle Mosaic Virus* was determined by analyzing signals coming from BERA biosensor and ANN (Glezakos et al., 2010). BERA provided time series data with a specific pattern for each virus; since information may be affected by external factors which can affect its performance and interactive preprocessing based on genetic algorithms was applied to the time series information. The obtained data was interpreted by ANN model to identify each virus variety and success rate of 0.899 was achieved.

4.6. Pharmaceutical applications

Evaluation of signal contour by ANN was used to quantify both penicillin concentrations and potassium ion concentrations with a single enzyme field effect transistor (En-FET) coupled with flow injection analysis (Hitzmann & Kullick, 1994). Penicillin G amidase was used as bioreceptor. The effect of ion concentration (5, 15, 25, 35 and 50mM) over a fixed penicillin concentration (1.5, 2.5, 5.0, 7.5 and 10 g/L) was recorded and 7 amplitude values (from 20 to 44 seconds in steps of 4 seconds) were used as ANN inputs. For architecture, three layers networks with 3, 5, 7 and 9 neurons in the hidden layer were tested. Penicillin showed a consisted quantification with average error of 5.4% and deviations of near 10%, while potassium ion concentration showed a lower performance with an error of 3.3% but deviations as high as 38.9%.This was one of the

first approaches using signal features from the same biosensor to perform multianalyte quantification.

4.7. Dedicated devices

The inclusion of automatic systems based on flow injection for liquid handling in conjunction with biosensor arrays and ANN is listed in this section. Main advantages of this approach are the full automation of the overall monitoring process, continuous operation and repeatability of the measurements and possibility of real time analysis.

Gutes *et al*. proposed the implementation of a Sequential Injection Analysis (SIA) System coupled with a voltammetric biosensor array for the determination of mixtures of glucose and ascorbic acid in fruit juices (Gutes et al., 2006). The array was composed by 3 biosensors with glucose oxidase enzyme immobilized over electrodes with different metallic catalyst (Au/Pd, Pt or Pd). Mixtures of glucose and ascorbic acid were automatically prepared by the SIA system and biosensors records (52 intensities) for each mixture were taken. Instead of using the whole records as ANN inputs record (52 intensities x 3 biosensors = 156 intensities), records from the three biosensors were added and baseline was subtracted; therefore ANN inputs were reduced to 52. A final architecture of 52 inputs, 3 neurons in the hidden layer with tansig transfer function and 2 neurons in the output layer with purelin transfer function was chosen accordingly a low RMSE of 0.4680. Correlations for this model were 0.9954 for glucose and 0.9791 for ascorbic acid in the testing test. Slopes and intercepts values were 1.015 ± 0.034 and $-2.3 \times 10^{-2} \pm 5.6 \times 10^{-2}$ for glucose while 0.915 ± 0.067 and $5.8 \times 10^{-3} \pm 5.6 \times 10^{-3}$ were obtained for ascorbic acid. After modeling, an automatic analysis with orange juice samples was performed. An acceptable performance glucose determination was obtained, with errors in a range of 0.87-12%. Determination of ascorbic acid was poorer as compared with glucose. This was attributed to the low concentration of ascorbic acid present in the samples.

A similar approach was used for the automatic determination of insecticides dichlorvos and methylparaoxon (Valdes-Ramirez, Gutierrez, del Valle, Ramirez-Silva, Fournier & Marty, 2009). As in previous studies, inhibition of AChE was used as analytical indicator of insecticide presence. In the first study, a multichannel Flow Injection System (FIA) was coupled with an enzymatic biosensor array for automatic liquid handling. Stocks of pesticides mixtures were dispensed by FIA system to the biosensor array and measurements of remaining enzymatic activity were taken. Generated data was modeled by ANN architecture of 3 input neurons, 3 neurons in the hidden layer with tansig function and 2 neurons in the output layer with purelin transfer function. Resulting slopes and intercepts from predicted values of ANN model were 0.98 ± 0.61 and 0.005 ± 0.035 for dichlorvos, while values of 0.91 ± 0.54 and 0.09 ± 0.60 were obtained for methylparaoxon. When tested with real water samples, recovery yields for dichlorvos was 104% and 118% for methylparaoxon. A similar system for *in-situ* analysis was developed by Crew *et al*. A multichannel flow system was coupled with an enzymatic biosensor array with diverse AChE varieties to obtain a distinctive inhibition pattern for organophosphate insecticides (Crew, Lonsdale, Byrd, Pittson & Hart, 2011). Calibration patterns for CFV, CPO, dichlorvos, malaoxon, chlorpyrifos methyl oxon and pyrimiphos methyl oxon were presented to the network for training. The system was applied to the detection of organophosphate insecticide presence in water and food samples by comparing the input pattern produced by biosensor array interaction with the sample with those obtained during calibration. When no significant differences from calibration were found, the absence of insecticide was assumed in both food and water. An alternative for portable instrumentation was presented by Alonso *et al*. with a hardware

implementation of an ANN in a low cost chip for insecticide quantification (Alonso, Istamboulie, Ramirez-Garcia, Noguer, Marty & Munoz, 2010). A study of networks configurations (number of layers, neurons in the hidden layer, training algorithm, etc) were previously tested in MATLAB. Two networks (ANN_1 and ANN_2) were selected and implemented in a dsPIC30F6010 microcontroller. Concentrations of CPO and CFV were evaluated with the trained model. The correlation coefficients obtained for the training test with ANN_1 model were 0.992 for CPO and 0.987 for CFV with a RAE of 4.40%. For the second model (ANN_2) correlations were 0.996 for CPO and 0.994 for CFV with a RAE of 0.23%. The time for system response was 948µs ANN_1 and 800µs for ANN_2 which can be considered a real-time application.

5. Conclusions

The applications and advances of biosensor monitoring coupled with ANN in different fields were presented. First applications were mostly focused on the improvement of single biosensor performance. Usually, signal preprocessing was null and feature extraction (when utilized) was based on user experience. The main goal was improved analytical performance trough a more sophisticated calibration tool. Even good results were obtained with this approach, highly attractive applications resulted from the coupling of biosensor arrays and ANN modeling. By exploiting higher input dimensionality, cross sensitivities and non specificities in biosensors (traditionally considered as undesirable features) multianalyte detection, determination of target analytes in presence of interfering species and quantification in complex samples were achieved by using ANN modeling. These advantages has been achieved with other multivariate calibration tools; however ANN exposed a superior performance when modeling non-linearities usually found in biosensors and biosensor arrays as compared with linear models. Improvements in biosensor design, bioreceptors, transducers and measurements techniques will provide signals with higher information that could be used to improved analytical performance of the exposed approach.

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