

Artificial neural networks study of the catalytic reduction of resazurin: stopped-flow injection kinetic-spectrophotometric determination of Cu(II) and Ni(II)

Diana M. Magni^a, Alejandro C. Olivieri^b, Adrian L. Bonivardi^{a,c,*}

^a *Departamento de Química, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santiago del Estero 2829, S3000AOM Santa Fe, Argentina*

^b *Departamento de Química Analítica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina*

^c *Instituto de Desarrollo Tecnológico para la Industria Química, Güemes 3450, S3000GLN Santa Fe, Argentina*

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Abstract

An artificial neural network (ANN) procedure was used in the development of a catalytic spectrophotometric method for the determination of Cu(II) and Ni(II) employing a stopped-flow injection system. The method is based on the catalytic action of these ions on the reduction of resazurin by sulfide. ANNs trained by back-propagation of errors allowed us to model the systems in a concentration range of 0.5–6 and 1–15 mg l⁻¹ for Cu(II) and Ni(II), respectively, with a low relative error of prediction (REP) for each cation: REP_{Cu(II)} = 0.85% and REP_{Ni(II)} = 0.79%. The standard deviations of the repeatability (s_r) and of the within-laboratory reproducibility (s_w) were measured using standard solutions of Cu(II) and Ni(II) equal to 2.75 and 3.5 mg l⁻¹, respectively: s_r [Cu(II)] = 0.039 mg l⁻¹, s_r [Ni(II)] = 0.044 mg l⁻¹, s_w [Ni(II)] = 0.045 mg l⁻¹ and s_w [Ni(II)] = 0.050 mg l⁻¹. The ANNs-kinetic method has been applied to the determination of Cu(II) and Ni(II) in electroplating solutions and provided satisfactory results as compared with flame atomic absorption spectrophotometry method. The effect of resazurin, NaOH and Na₂S concentrations and the reaction temperature on the analytical sensitivity is discussed.

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1. Introduction

Kinetic-based methods have been continuously growing during the last decades, together with automated techniques such as flow injection analysis (FIA), which have gained special analytical interest because of their simplicity, feasibility, high sampling frequency and low cost of reagents and samples [1,2]. In order to take advantage of the performance of the flow injection (FI) systems, alternative modes to the conventional systems have been proposed including, in the

case of kinetics processes, stopped-flow techniques which increase the residence time and thus the conversion of the measured species [3,4]. The usual procedure is to stop the flow before reactions have been completed, to monitor the time-dependent signal as the reaction continues in the detection cell, and to use the resulting response profile to determine analyte concentrations. The response profile is a data vector from each of several standards, which can be arranged into a two-way matrix, and correlated to analyte concentrations using first-order multivariate calibration methods [5,6].

When the data structure is intrinsically non-linear, classical calibration methods cannot be applied, because their underlying models are linear. This kind of problem arises, for example, when the analyte acts as the catalyst of a kinetic

* Corresponding author. Tel.: +54 342 4571164x2542; fax: +54 342 4571162.

E-mail address: bonivar@fiqus.unl.edu.ar (A.L. Bonivardi).

reaction [7]. In these cases, an excellent alternative is the use of artificial neural networks (ANNs) [8,9]. These algorithms are based in concepts loosely related to the behavior of the human brain: the variables are assigned to mathematical objects called neurons, and a mathematical function is associated with the so-called intra-neural connections. A neural network model is composed of a large number of simple processing elements or neuron nodes, organized into a sequence of layers [8]. ANNs mathematically transform an input vector (a vector of variables assigned to a number of neurons) into an output vector through a suitable transfer function. Neural networks show several advantages: they do not require prior knowledge of the model-related function, leading to better generalizations because they are able to model complex relationships. Their flexibility has been a decisive feature compared with parametric techniques that require the assumption of a specific model form. In addition, ANNs avoid the time-consuming and possibly expensive task of deterministic model identification required for kinetic analytical studies. They can be adequately trained to produce quantitative results; in the field of analytical chemistry the back propagation model is the most frequently employed. Basic theory and application to chemical problems of back-propagation ANNs can be found in the literature [8,9]. Another relevant use of ANNs is in optimization of analytical methodologies [10–13].

Kinetic methods coupled to ANNs have been previously applied for analyte determinations in mixtures. Pertinent examples are the oxidative coupling reaction of *N,N*-diethyl-*p*-phenylenediamine with chlorophenol derivatives [14], the simultaneous determination of methanol and ethanol in a spectrophotometric enzymatic system [15], the kinetic spectrophotometric determination of acetaminophen and phenobarbital [16], the simultaneous determination of V(IV) and Fe(II) as catalysts in the redox reaction of bromate with methyl orange [17] and the resolution of Co(II) and V(IV) mixtures [18]. The comparison of the ANN results with those provided by other chemometric techniques such as principal component regression (PCR) or partial least-squares (PLS) favors the former, such as in the analysis of mixtures of Fe(III), Co(II) and Zn(II) by displacement from their EGTA complexes with 4-(2-pyridylazo)resorcinol (PAR) [19], and in the resolution of ternary mixtures of antioxidants [20].

Another interesting example is the determination of mixtures of heavy metal ions at high concentrations in industrial samples. Ni et al. have reported the simultaneous spectrophotometric determination of Co(II), Ni(II), Cu(II), Fe(III) and Cr(III) in electroplating bath solutions by the inclusion of ethylenediaminetetraacetate (EDTA) solution as chromogenic reagent [21]. They built calibration models based on different chemometric models, including ANNs, in the latter case with good analytical performance.

The determination of Cu(II) and Ni(II), among other metal ions, is very important for the electroplating industry of our region. The quality of electroplated products depends partly on the composition of the metal ions in the electroplating

bath, and thus their analyses allow to maintain the needed chemical composition to achieve the required product. On the other hand, these determinations allow for an accurate control of wastewater quality.

The aim of the present study is the optimization and ANN calibration of a kinetic methodology using a stopped-flow injection system for the determination of Cu(II) and Ni(II), based on their catalytic effects on the reduction reaction of resazurin by sulfide. This reaction has already been proposed for the determination of Se(IV) [22] and Pb(II) [23] using batch procedures in both cases. A recent study employs the same chemical system for the determination of Pb(II) with a stopped-flow injection technique [24]. Both batch and on-line analytical procedures were based on pseudo first-order kinetic assumptions. Thus, linear relationships were assumed between the catalyst concentration and: (1) the absorbance changes for a fixed reaction time or (2) the time needed to obtain a fixed absorbance change. This methodology was found to work well for the determination of analytes at low concentration levels. In the present report, we found that the catalyzed reaction mechanism did not follow a pseudo first-order kinetic model for the investigated cations, and that significant non-linear effects were present. These effects did not allow for a successful calibration at high concentrations using linear relationships, and therefore ANNs were applied.

2. Experimental

2.1. Apparatus and flow injection manifold

Spectrophotometric UV–vis measurements were performed using a Perkin Elmer UV Lambda 20 spectrophotometer, equipped with a 32 μ l flow quartz cell supplied by Wilmad. A personal computer for time recording and data acquisition was used.

The flow injection manifold used is depicted in Fig. 1. An 8-channel Ismatec-IPC peristaltic pump (ISMATEC-IPC, Switzerland) fitted with Tygon[®] tubes was used to pump all solutions and to provide the stopped-flow. A 6-port Vici-Cheminert C22Z (VALCO, USA) injection valve was used to inject the sample into the water carrier stream. A 4-port Vici-Cheminert C22Z (VALCO, USA) injection valve was used to perform the system cleaning operations. All the manifold tubing was made of PTFE (0.5 mm i.d.).

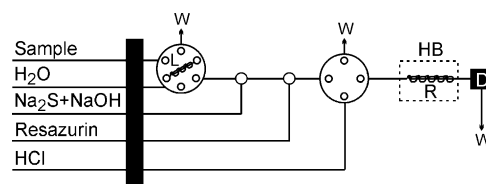


Fig. 1. FI manifold for the spectrophotometric determination of Cu(II) and Ni(II) with Resazurin. L: sample loop, HB: heated water bath, R: knotted reactor, D: detector, W: waste.

A conventional heated water bath was used to keep the knotted mixing reactor at a constant temperature.

2.2. Reagents

All the employed chemicals were of analytical-reagent grade and solutions were prepared using deionized water.

A resazurin stock solution was prepared by dissolving 0.1100 g of resazurin (Sigma, purity 90%) in a 100 ml volumetric flask with water. The working resazurin solutions were made by appropriate dilution of the stock solution, which was stable for several months.

The sodium sulfide working solutions were made by dissolving the appropriate weight of sodium sulfide hydrate (Merck, about 35% Na₂S) with water. These solutions had to be freshly prepared everyday.

The stock sodium hydroxide solution was prepared by dissolving 40 g of sodium hydroxide (Merck, purity 99%) in 100 ml of water. The appropriate volumes of this solution were added to the sodium sulfide working solutions.

Standard stock solutions of copper and nickel were prepared by dissolving 0.3929 g of copper sulphate pentahydrate (Merck, purity 99%) and nickel sulphate heptahydrate (Merck, purity 99%) in a 100 ml volumetric flask each. Working solutions of both metal ions were prepared by appropriate dilution of the stock solutions.

2.3. Glassware

All the glassware used was cleaned with detergent, rinsed with tap water followed by deionized water, soaked overnight in a nitric acid (15%, v/v) bath and carefully rinsed with deionized water before use.

2.4. Procedure

Before pumping the reagents, the whole FI system was cleaned by pumping 1:1 hydrochloric acid solution during 30 min and deionized water for additional 30 min.

The water carrier and reaction components, sodium sulfide (in a sodium hydroxide medium) and resazurin solutions, were all transported at the same flow rates through their respective channels to the point where they were mixed. The mixture passed through a knotted mixing coil (250 cm length \times 0.5 mm i.d.) placed in the heated water bath to allow the emergent solution to reach the flow-cell completely mixed and with a uniform temperature. Reagents were pumped through the manifold until the resazurin absorbance was stable at a maximum value. When this latter condition was reached, 250 μ l of the sample solution containing Cu(II) or Ni(II) were injected into the carrier. After 60 s, the flow was stopped for 120 s. During this time the reaction took place in the flow-cell and the conversion degree of resazurin was monitored spectrophotometrically at $\lambda_{\text{abs}} = 605$ nm (slit width = 2 nm). The kinetic data were acquired at a rate of 1 s per point and recorded in the computer. The reaction window

of 120 s was chosen as a compromise between high instrumental signal and adequate analytical frequency.

Once the preset reaction time had elapsed, the pump was turned on and the cleaning operation was performed, first with hydrochloric acid solution and then with deionized water. This cleaning step is of great importance to remove metal ions away from the quartz cell. Finally, the system was ready for a new sample injection.

3. Methods

3.1. Experimental design

The experimental parameters of the FIA assembly that could affect the dispersion of the signal were optimized by changing each variable in turn, while keeping all the other ones constant. The variables selected for optimization were the mixing reactor length, the flow rate of reactant stream and the sample volume size.

The influence of physicochemical variables such as concentrations of reactants and temperature of mixing coil was studied using an orthogonal design [25], in which all the factors investigated were varied simultaneously. In this design, five levels for each factor were selected and there were a total of 25 experiments. The response selected to evaluate the design results was the absorbance change (ΔA) along 120 s of reaction time. The details of the factor design are shown in Table 1. The optimum conditions of each of the factors were evaluated using a response surface optimization method, implemented in the Statgraphic 3.0 statistical package.

3.2. Kinetic simulation methods

The Statgraphic 3.0 statistical package and routines written for the Matlab 5.3 environment were used to predict kinetic parameters for different kinetic models.

3.3. Artificial neural networks

In modeling the current calibration problem, a neural network trained by back-propagation of errors was used. The latter technique is attractive because of the well-defined and explicit set of equations for weight corrections. These equations are applied throughout the layers, beginning with the correction of the weights in the last (output) layer, and then continuing backwards towards the input layer.

The architecture of the network (number of layers, number of neurons in each layer and neural connection mode) is the main feature influencing the flexibility of the ANN model. Before the learning process begins, an initial architecture of the neural network must be chosen and then modified during the learning or testing phase. A trial and error methodology was employed to find the best architectures for all ANNs used in this work. Two parameters must be defined for each architecture: the learning rate constant η and the momentum

Table 1
Factor levels in orthogonal factorial design and their optimum values for Cu(II) [6 mg l⁻¹] and Ni(II) [15 mg l⁻¹]

Factors	Analyte	Levels					Optimum
Temperature (°C)	Cu(II)	34	36.5	39	41.5	44	34
	Ni(II)	34	36.5	39	41.5	44	44
Resazurin (mg l ⁻¹)	Cu(II)	25	30	35	40	45	45
	Ni(II)	25	30	35	40	45	45
Na ₂ S (% w/v)	Cu(II)	0.2	0.8	1.4	2.0	2.6	1.4
	Ni(II)	0.2	0.4	0.6	0.8	1.0	1.0
NaOH (mol l ⁻¹)	Cu(II)	0	0.3	0.6	0.9	1.2	0.4
	Ni(II)	0	0.2	0.4	0.6	0.8	0

constant μ . The former determines the speed at which the weights change, and represents a reasonable trade-off between fast learning and converging to the lowest minimum. The momentum constant gives the learning process a certain capacity for inertia, enabling the network to avoid partial minima. Both parameters are closely related, and they were simultaneously varied to get their optimum values for calibration purposes.

The root-mean-square (RMS) error between the targets and the output (ANNs predicted values) was used as the parameter to stop the ANNs training process

$$\text{RMS} = \sqrt{\frac{\sum (C_{\text{nom}} - C_{\text{pred}})^2}{I - 1}} \quad (1)$$

where C_{nom} and C_{pred} are the nominal and predicted concentrations of a test group of samples respectively, and I the number of samples.

An ANN routine developed in the Matlab 5.3 environment was applied, performing calibration and prediction using the kinetic profiles recorded by the experimental method described above.

4. Results and discussion

4.1. Chemical system

The reduction reaction of resazurin by sulfide in alkaline solution proceeds slowly without added catalysts. It is known that the reduction rate increases significantly in the presence of trace amounts of certain metal ions. The complete kinetic mechanism for the reaction has not been reported. Afkhami et al. have proposed that the reaction takes place in two steps (see Fig. 2) [23]: the first one, whose product is resorufin, is assumed to be irreversible, but the second one, the reduction of resorufin to dihydroresorufin, is assumed to be reversible and fast (i.e., both resorufin and dihydroresorufin are in equilibrium). These authors also proposed that only the first step is catalyzed by metal ions. However, our absorbance-time profiles for both Cu(II) and Ni(II) (Fig. 3) clearly suggests the presence of an additional, autocatalytic step by a reaction

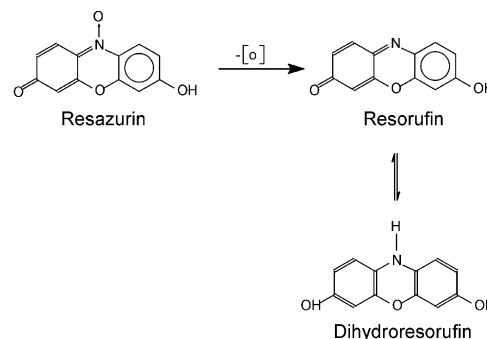


Fig. 2. Resazurin reduction reaction.

product [26]. This indicates that the assumption of a first-order kinetics for our reaction system is unfeasible, and that a non-linear response is expected for the determination of the Cu(II) and Ni(II) analytes.

4.2. Effect of experimental variables

The variables affecting the performance of the proposed kinetic method for the determination of both Cu(II) and Ni(II) were optimized. The FI variables involve the ability to mix sample and reactants solutions automatically and the acquisition of kinetic data from the mixed solution. The

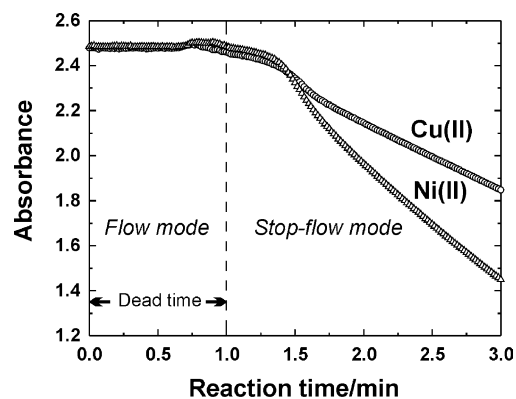


Fig. 3. Absorbance-time profiles for standard solutions: 1.0 mg l⁻¹ of Cu(II) and 2.0 mg l⁻¹ of Ni(II), as indicated.

physicochemical variables include the concentrations of reagents and the temperature of the mixing reactor that lead to a maximum in the signal changes.

4.2.1. FI variables

The mixing reactor length was varied between 50 and 500 cm for the higher flow rate used in this work (3.4 ml min^{-1}). The shortest reactor length that allowed a complete mixture of the reactants (250 cm) was chosen. For shorter reactor lengths the mixing process was deficient and the signals were noisy and not reproducible.

When using a stopped-flow technique, the flow rate and the required time to stop the pump after the sample injection are of great importance. In order to select these parameters a standard tartracine solution was used (absorbance = 1.30 at $\lambda = 428 \text{ nm}$). Water was pumped in all reagent channels (the flow rate was varied between 0.8 and 3.4 ml min^{-1}) and the sample loop was filled with the tartracine solution. The same flow rates and their corresponding stop times were verified for our chemical system and a total flow rate equal to 1.1 ml min^{-1} was selected. For this total flow rate, one minute was necessary to stop the pump after the loop injection. For lower rates the reaction began in the mixing reactor and for faster rates the noise of the system increased.

The influence of sample volume was studied in a range of 100 – $500 \mu\text{l}$ using the said tartracine solution. It was observed that, for a volume of $250 \mu\text{l}$, there is a period of 10 s where the dispersion coefficient is reasonably good ($1.05 \leq D \leq 1.08$) to perform our experiments. The maximum signal is wide enough to obtain reproducible results, minimizing stop time errors. With smaller loops the dispersion coefficient is larger, and with larger ones the improvement for the dispersion coefficient is not significant and the required time to fill the loop is too long. Therefore, an injection loop of $250 \mu\text{l}$ was selected, and the pump was stopped 60 s after the sample was injected.

4.2.2. Physicochemical variables

The selected factors were the concentration of resazurin (C_R), the concentration of sulfide (C_S), the concentration of sodium hydroxide (C_{NaOH}) and the temperature of the bath where the mixing reactor was placed (T).

A set of working intervals was selected from a series of preliminary experiments which take into account certain operative limits. In these intervals, the orthogonal design (see Section 3.1 and Table 1) was used to obtain an analytical system that would provide greater sensitivity. Our task was to seek the four input values: C_R , C_S , C_{NaOH} and T corresponding to a maximum output value of decrease in resazurin absorbance (ΔA) for a fixed reaction time. For this purpose, we employed a surface response optimization procedure.

The design analysis for Cu(II) shows that two factors were significant (C_R and T) at the 95% confidence level, that the model explains 88% of the total variability and that no correlation between variables was found. An increase of the analytical signal by increasing resazurin concentration is to be

expected for catalytic, pseudo first-order reactions, however a higher limit is imposed by the increase of the signal noise due to a decrease in the detection capability of the photomultiplier tube for high absorbance values. Consequently a resazurin concentration equal to 45 mg l^{-1} was selected as the optimum concentration value. Although the temperature is known to have a positive effect in kinetic-based determinations, the resulting negative effect of this factor on the signal can only be explained from the coexistence of unknown side reactions favoring the consumption of the analyte. Therefore, for this study we chose the lower value obtained as optimum (34°C). Although C_S and C_{NaOH} showed no statistical significance, the response surface analysis for these two factors enabled us to select their optimums: 1.4% (m/v) and 0.4 mol l^{-1} for C_S and C_{NaOH} , respectively.

The response surface analysis for Ni(II) shows that three factors (C_R , C_S , C_{NaOH}) were significant for the same confidence level. The model explains 94% of the total variability and no correlation between variables was found. The effect of resazurin concentration is similar to that for Cu(II), allowing to arrive at the same conclusions. The analytical signal increases with an increase of sulfide concentration, but the highest limit is imposed by the undesirable on-line precipitation of NiS. The NaOH concentration has a negative effect in the Ni(II)-catalyzed reaction, and the possible range for this variable study was limited due to a precipitate formation. This behavior is attributed to the low solubility product of $\text{Ni}(\text{OH})_2$ and the consequent removal of Ni(II) ions from the reaction medium for the Ni(II) concentrations used in this study. The optimum signal was found in the absence of NaOH, but a concentration of 0.2 mol l^{-1} of NaOH was selected for the rest of the study in order to improve the sulfide solution stability. The factor temperature was not significant at 95% confidence level, but a positive sign was observed on the response, and therefore the higher value (44°C) was adopted as desirable.

It is difficult to explain, from a thermodynamic point of view, our operative limits for both cations in studying sulfide and hydroxide effects. For Ni(II) we attributed the precipitate formation to the low solubilities of $\text{Ni}(\text{OH})_2$ and NiS. However, in the case of Cu(II) we expected a lower working range for both studied factors if only thermodynamic aspects were taken into account. A possible explanation for our more extended experimental working range in the case of Cu(II) is the formation of Cu(II) soluble species in alkaline medium [27], and this reaction can compete kinetically with the formation of other less soluble species.

4.3. Analytical features

4.3.1. Kinetic calibration

We have already pointed out in Section 4.1 that a pseudo-first-order kinetic mechanism cannot be employed to explain the absorbance versus time profiles shown in Fig. 3. In the stopped-flow mode, i.e. after 1 min for reaction time, and using low concentration of any metal cation, the value of the

Table 2
Concentration and scaled PC's scores of the training and test sets for Cu(II) and Ni(II)

Cu(II) concentration (mg l ⁻¹)	PC1	PC2	PC3	PC4	Ni(II) concentration (mg l ⁻¹)	PC1	PC2	PC3
Training set								
0.75	-1	-1	-1	0.1986	1.00	-1	-1	-0.9911
1.50	-0.7240	-0.3014	1	-1	1.50	-0.7208	0.1795	1
2.00	-0.6070	0.0034	0.7911	0.6469	2.00	-0.4003	0.9286	0.0836
2.50	-0.4695	0.3513	0.3655	1	4.00	0.3155	1	-1
3.00	-0.3040	0.7624	0.0260	0.7194	7.00	0.7139	0.3498	-0.3335
3.50	-0.2237	0.8791	-0.27	-0.1125	9.00	0.7832	0.1030	-0.1786
4.00	-0.1249	1	-0.4733	-0.6749	11.00	0.9057	-0.2920	-0.0423
6.00	1	-0.7788	0.0991	0.1589	15.00	1	-0.7834	0.2538
Test set								
0.50	-1.1228	-1.5689	-1.1717	4.5459	2.50	-0.1385	1.0428	-0.6488
1.00	-0.9239	-0.7561	-0.1899	-1.6187	3.00	0.0597	1.1897	-0.9227
5.00	0.1601	1.4153	-0.5493	-1.9391	5.00	0.5592	0.7397	-0.7393

absorbance was almost constant for a short time and then the signal started to decrease. This initial “induction” period is typical of autocatalytic reactions where the global reaction is catalyzed by a reaction product: at the beginning the decomposition rate of the reagent is slow but as long as the product is formed the reagent decomposition is accelerated [26]. It is obvious then that a step where resazurin reacts with a product have to be involve in the whole kinetic mechanism.

Thus, we include an additional parallel step to the mechanism shown in Fig. 2: the reaction between resazurin and resorufin, to evaluate and to fit the new model with the experimental profiles obtained for different concentrations of catalyst in the range of 0.5–6 mg l⁻¹ for Cu(II) and 1–15 mg l⁻¹ for Ni(II) using the software packages already described in Section 3.2.

Although good fittings between predicted and experimental data were obtained when using the new additional step in the mechanism of Fig. 2, it was not possible to arrive to useful analytical relations for the determination of the studied metal cations.

We also tested a less complex mechanism eliminating the formation of dihydroresorufin from the new one. However, the theoretical kinetic model did not fit properly the experimental data and a non-linear relationship was found between the Ni(II) concentration and the kinetic constant for the first step of the mechanism. Only Cu(II) concentration could be linearly correlated to that kinetic constant.

We concluded that a more rigorous study on the reaction kinetic must be performed to explain the results in terms of deterministic models that allow us the analytical determination for the catalysts.

4.3.2. ANNs calibration

In view of the difficulties in obtaining the underlying kinetic mechanism which would allow us to solve the analytical problem on a deterministic basis, a multivariate, non-linear calibration was performed using ANNs. For this purpose, two sets of eleven calibration samples each were used for training

the network for each analyte. The sets ranged from 0.5 to 6 and from 1 to 15 mg l⁻¹ for Cu(II) and Ni(II), respectively. They were randomly divided into a training set (70% of samples) and a test set (30% of samples) (see Table 2). The profiles obtained using the methodology explained above for these samples were first compressed by principal components preprocessing, and then used as input of an ANN trained by back propagation of errors. Each column of the input matrix consisted of 120 data points for each profile corresponding to absorbance data acquired during a reaction period between 60 and 180 s. In this way, we obtained matrices composed of 11 columns and 120 rows as input data, which after principal component compression, yielded matrices composed of 11 columns and a number of rows equal to the number of input neurons (see scores in Table 2). The output layer was a single node corresponding to the analyte concentration, so that the output vectors were those corresponding to calibration concentrations for Cu(II) and Ni(II), respectively.

The parameters used to build the ANN models were optimized (see Table 3). Different combinations of learning rates and momentums were assayed. Learning rates of 0.5 and 0.4 were found to work well with the data sets for Cu(II) and Ni(II), respectively. While learning rates were being investigated, momentum values were also varied to find a ratio for

Table 3
Optimized parameters used for the construction of ANN models

Parameters	Analyte	
	Cu(II)	Ni(II)
Input nodes	4	3
Hidden nodes	2	2
Output nodes	1	1
Learning rate	0.5	0.4
Momentum	0.5	0.6
Input layer transfer function	Linear	Linear
Hidden layer transfer function	Sigmoid	Sigmoid
Output layer transfer function	Sigmoid	Sigmoid
Number of iterations	4184	455

Table 4
RMS for calibration after applying different regression models

Model	Cu(II)			Ni(II)			
	PCR	PLS	ANN	PCR	PCR	PLS	ANN
Region (number of sensors)	1–120	90–120	1–120	1–120	80–110	80–110	1–120
Factors	3	1	4:2:1 ^a	2	3	3	3:2:1 ^a
RMSC (mg l ⁻¹)	0.095	0.143	0.037	1.223	0.456	0.456	0.350

^a ANN architecture.

the relative combination of the two parameters that would give the faster optimization of the networks. Momentums of 0.5 and 0.6 were selected for Cu(II) and Ni(II), respectively.

The selected transfer function was of a sigmoid type, since it is very versatile and appropriate for non-linear systems. This kind of function was used in the hidden and the output layers.

The proper number of nodes in the input and hidden layer was investigated by trial and error by training ANN with different architectures. The parameter used to evaluate the different ANNs performance was the RMS value obtained for the test sample set. For Cu(II) the minimum of RMS occurred for four input nodes when it was varied from 3 to 6. Varying the number of nodes in the hidden layer from 2 to 6, for four nodes in the input one, we found that the minimum RMS was given by two nodes in the hidden layer. To select the best number of training epochs, error curves of RMS values versus training epochs were built when the optimum architecture was used. The minimum for RMS was detected at 4184 epochs and the relative error of prediction was equal to 0.85%.

The minimum of RMS for Ni(II) occurred when three and five nodes were used for input layers. For the two alternatives the number of nodes in the hidden layer was varied from 2 to 5. According to its generalization ability on the testing set, curves of the RMS versus the number of hidden layer nodes were made. When the number of hidden nodes was equal to 2, for three nodes in the input layer, RMS was minimized. Analogous curves for five nodes in the input layer let to larger RMS values. Hence three and two nodes were adopted for input and hidden layers, respectively. The minimum for the RMS values curves was detected for 455 epochs and the relative error of prediction was equal to 0.79%.

Despite of the inherent non-linearity of these kinetic data, we also evaluated different regression linear models to verify whether ANNs results are better than those obtained from the linear models. We first used PCR models with the same PCs as in the case of ANNs for both cations, Cu(II) and Ni(II). After applying a forward selection procedure only three and two components, respectively, were significant for these linear models. A moving window strategy was applied to find the most informative range of data, finding only an improvement of the results for the Ni(II) analyte. In the case of Cu(II), the full range of data gave the best results. We also applied PLS models optimized by the moving window strategy, using the cross-validation procedure to assist in the selection of the

number of factors. For comparison purposes, Tables 4 and 5 show the results of the RMS values of the calibration (RMSC) and the predicted concentration values, respectively, for the different regression models. In all the cases, ANNs methodology gives the best analytical results, as expected.

Fig. 4 summarizes the predicted concentrations by the best ANNs versus nominal concentrations for calibration and test sets for Cu(II) and Ni(II), respectively.

4.3.3. Predictions for the validation sets

The optimal ANN calibrations found for each analyte were applied to the prediction of the concentrations of the components in six synthetic samples for both Cu(II) and Ni(II), corresponding to two validation sets. For this purpose, triplicate measurements of each sample were made using our method, thus the resulting profiles were evaluated by ANNs. Triplicate determinations were also performed for the validation sets using flame atomic absorption spectrophotometry (FAAS). These results are collected in Table 6 for both Cu(II) and Ni(II).

A convenient way to establish whether bias is absent or not for the determination in both cases is to draw the elliptic joint confidence region (EJCR) for the slope and intercept when plotting $C_{\text{pred,ANNs-kin}}$ versus $C_{\text{pred,FAAS}}$ [28]. Fig. 5 shows these regions for the determination of Cu(II) and Ni(II), respectively. As can be seen, both ellipses contain the theoretically expected value of (1, 0), indicating that the method is accurate.

4.3.4. Predictions on real samples

The present methodology was applied to two electroplating solutions from a local industry containing Cu(II) or Ni(II).

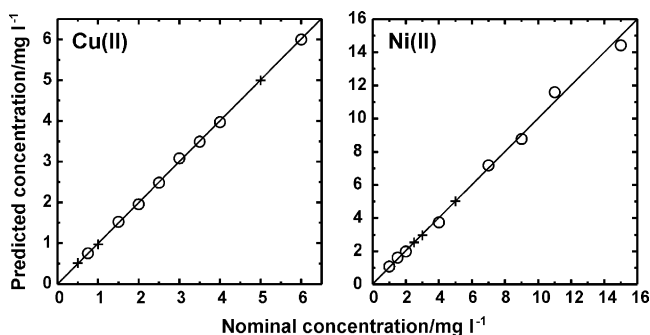


Fig. 4. ANNs predicted concentrations: (○) calibration set and (+) test set for Cu(II) and Ni(II), as indicated.

Table 5
Predicted concentration values of Cu(II) and Ni(II) for training and test sets after applying different regression models

Cu(II) (mg l ⁻¹)				Ni(II) (mg l ⁻¹)				
Actual	PCR (1–120) ^a	PLS (90–120) ^a	ANN (1–120) ^a	Actual	PCR (1–120) ^a	PCR (80–110) ^a	PLS (80–110) ^a	ANN (1–120) ^a
Training set								
0.75	0.76	0.62	0.75	1.00	2.15	1.10	1.10	1.08
1.50	1.47	1.62	1.52	1.50	0.72	1.54	1.54	1.62
2.00	1.94	2.06	1.96	2.00	0.63	1.59	1.59	1.99
2.50	2.52	2.57	2.48	4.00	4.56	4.52	4.51	3.74
3.00	3.19	3.17	3.08	7.00	8.52	7.36	7.35	7.18
3.50	3.50	3.43	3.49	9.00	9.56	8.14	8.13	8.77
4.00	3.85	3.74	3.97	11.00	11.28	11.36	11.35	11.59
6.00	6.01	6.04	6.00	15.00	13.09	14.90	14.89	14.42
Test set								
0.50	0.22	0.12	0.51	2.50	1.84	2.26	2.26	2.54
1.00	0.94	0.90	0.97	3.00	2.60	3.08	3.07	2.96
5.00	4.80	4.64	4.99	5.00	6.63	6.16	6.16	5.02

^a Region (number of sensors).

Table 6
FAAS and ANNs-kinetic method results for the validation sets for Cu(II) and Ni(II)

Analyte	FAAS ^a (mg l ⁻¹)		ANNs-kinetic method ^a (mg l ⁻¹)	
	Average concentration ($C_{\text{pred,FAAS}}$)	Standard deviation	Average concentration ($C_{\text{pred,ANNs-kin}}$)	Standard deviation
Cu(II)	0.53	0.016	0.59	0.020
	1.34	0.002	1.26	0.024
	2.20	0.012	2.22	0.018
	2.74	0.014	2.64	0.036
	4.63	0.014	4.44	0.051
Ni(II)	1.20	0.006	1.10	0.012
	1.70	0.013	1.52	0.014
	2.05	0.020	2.33	0.027
	2.70	0.038	2.79	0.042
	3.63	0.025	3.56	0.038
	4.51	0.045	4.53	0.108

^a $n = 3$.

A preliminary dilution step of each solution was needed for the analysis of these analytes. Each diluted sample spiked to 1.5 mg l⁻¹ for Cu(II) and 2.5 mg l⁻¹ for Ni(II) was also analyzed. The results were compared with those obtained using FAAS technique as a reference method. Table 7 shows the excellent recovery results for ANNs-kinetic method and the t -values indicate that there are not statistical significant

differences between the concentration values determinate for both methodologies and analytes, at the 5% level.

4.3.5. Repeatability and reproducibility studies

In order to determine the method repeatability and the within-laboratory reproducibility as a measure of intermediate precision some guidelines provided by the

Table 7
Determination of Cu(II) and Ni(II) in electroplating bath samples

Analyte	Sample ^a	FAAS ($n = 4$)		ANNs-kinetic method ($n = 4$)		t
		Average (mg l ⁻¹)	R.S.D. (%)	Average (mg l ⁻¹)	R.S.D. (%)	
Cu(II)	Dilute sample ^b	0.700	1.60	0.686	2.90	1.227
	Spiked dilute sample ^c	2.19	0.27	2.24	2.40	1.875
	Recovery (%)	99.3		103		
Ni(II)	Dilute sample ^b	2.71	3.02	2.76	6.09	0.536
	Spiked dilute sample ^c	5.21	1.04	5.24	2.06	0.493
	Recovery (%)	100		99.2		

^a From an electroplating bath solution supplied by Cromados S.A. Company.

^b Dilution factor equal to 1:40,000 for Cu(II) and 1:20,000 for Ni(II).

^c Sample spiked to 1.5 mg l⁻¹ for Cu(II) and 2.5 mg l⁻¹ for Ni(II).

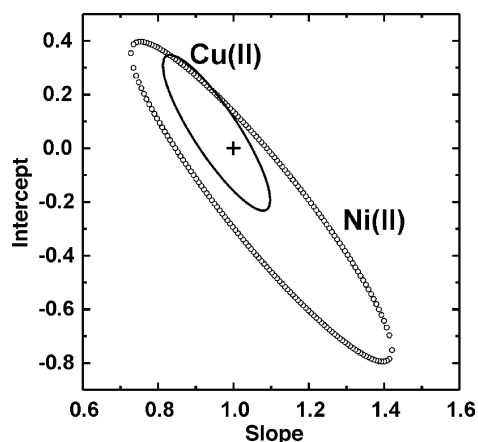


Fig. 5. Elliptic joint confidence region for the slope and intercept of the regression of predicted vs. nominal concentrations. The cross marks the theoretical (1, 0) point.

official literature were followed [29]. The basic experimental setup consisted in measuring five replicates during three consecutive days on a standard solution of analyte. Standard solutions containing 2.75 and 3.5 mg l⁻¹ for Cu(II) and Ni(II), were respectively chosen.

The ANOVA analysis for Cu(II) shows that the residual mean squares (s_r^2), named the mean squares within-days, is equal to 0.0015 mg² l⁻² and represents the repeatability conditions. The within-laboratory reproducibility, s_{wr}^2 , is equal to $s_r^2 + s_{\text{between}}^2$, where s_{between}^2 represents the variance due to the between-day effect and it was determined using the following expression:

$$s_{\text{between}}^2 = \frac{MS_{\text{between}} - MS_{\text{within}}}{n_j} \quad (2)$$

where j is the number of replicates for each day. Thus, s_{wr}^2 for Cu(II) was computed to be 0.0020 mg² l⁻². The conclusion is that the repeatability standard deviation is 0.039 mg l⁻¹ and the within-laboratory reproducibility standard deviation is 0.045 mg l⁻¹.

The same ANOVA analysis for Ni(II) led to a repeatability standard deviation equal to 0.044 mg l⁻¹ and a within-laboratory reproducibility standard deviation equal to 0.050 mg l⁻¹.

4.3.6. Analytical frequency

In the stopped-flow methodology employed in this work, the sample remains into the flow cell for a period long enough to produce contamination of the internal walls of our quartz cell. It is well known that metal ions can be easily and rapidly adsorbed only on deprotonated silanol surface groups (i.e. under basic conditions) [30]. These cations [e.g. Cu(II) or Ni(II)] can accumulate along successive runs, and were responsible of the drift of the analytical signal detected in this work when no cell cleaning process was applied. Therefore, a cleaning step involving an acid solution able to regenerate silanol groups is necessary after each determination, in order

to remove the cations adsorbed over the inner wall surface of the quartz cell. This cleaning process is very time-consuming and defines the analytical frequency of the method. We found that a HCl (1:1) solution was able to remove both Cu(II) and Ni(II) in 1.5 and 4.5 min, respectively. After the latter periods, a rinsed step of 1 min was added for both cations. In this way, the analytical frequencies for Cu(II) and Ni(II) were in 8 and 6 samples h⁻¹, respectively.

5. Conclusions

We developed a method based on the alkaline reduction of resazurin by sodium sulfide for the catalytic determination of Cu(II) and Ni(II), using a combination of stopped-flow injection analysis, molecular absorption spectrophotometry and neural network data processing. The method is simple, inexpensive, and reliable. It can be applied for the routine analysis of Cu(II) and Ni(II) using inexpensive instruments.

The use of a neural network approach proved to be an excellent tool for the optimization and calibration processes without the use of deterministic models for the kinetic mechanism of that complex reaction involving catalytic metal ions.

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