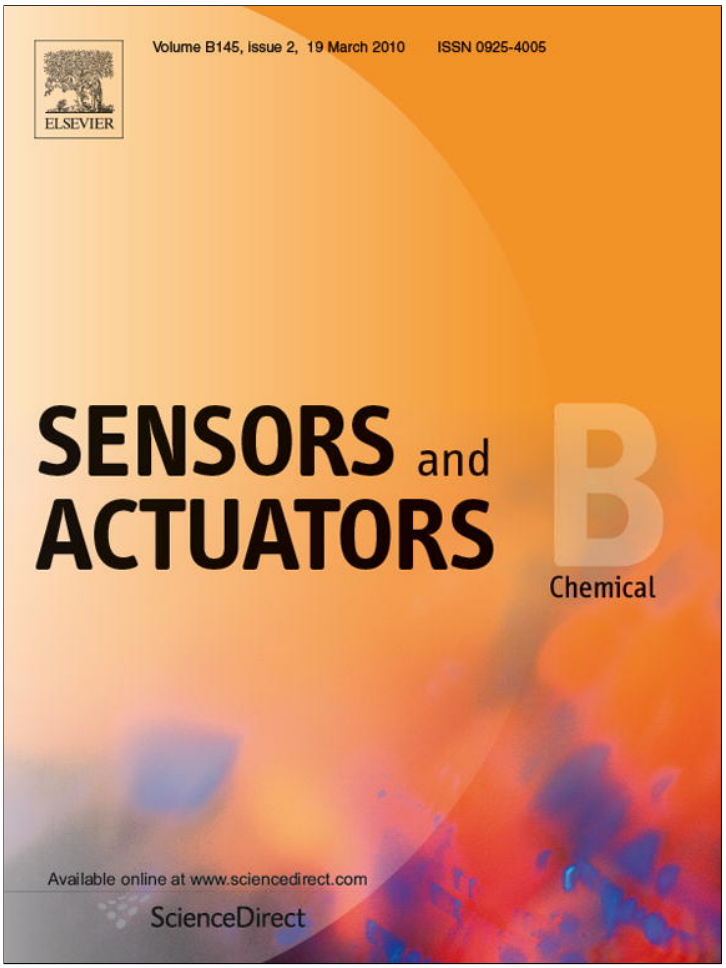


Sensors and Actuators B 145 (2010) 726–733

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Chelating electrodes as taste sensor for the trace assessment of metal ions

Jorge Yánez Heras, Silvio D. Rodriguez, R. Martín Negri, Fernando Battaglini *

INQUIMAE - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, C1428EHA Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 12 October 2009
Received in revised form 14 January 2010
Accepted 15 January 2010
Available online 25 January 2010

Keywords:
Chelating electrode
Trace metal detection
Electronic tongue
Impedance
Principal component analysis

ABSTRACT

Impedance spectroscopy combined with principal component analysis allows the trace detection of metal ions. The detection system is based on two modified electrodes, each of them containing a chelating agent (pyrocatechol violet and a nitrilotriacetic derivative); as the chelator is able to capture the metal ion at very low concentrations, important electrical changes are produced on the surface environment, generating patterns with different features for each ion. The system is able to differentiate eight metal ions (Al^{3+}, Fe^{3+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Ca^{2+} and Ag^{+}) at micromolar levels in ultrapure water. The method allows the detection of metal ions in aqueous samples without the need of sample conditioning, rinsing steps or the addition of probes. Selecting the appropriate frequencies and sensors, the array can also be applied to different aqueous systems such as bottled mineral water or concentrated NaCl (27%) yielding similar results.

1. Introduction

The assessment of hazardous metal ions is an important practice due to their potential toxic effects on humans, and/or the adverse impacts on the environment. The toxicity concern includes also aluminum, the most abundant metal in the Earth's crust, and the third most abundant element therein. Even though a direct relation between the role of aluminum and other metals (Cu, Fe) in Alzheimer's disease has not yet been definitively demonstrated, epidemiological evidence suggests that elevated levels of them in the brain may be linked to the development of the disease [1,2]. Currently, the U.S. EPA does not regulate aluminum under its drinking water program, but has a secondary non-enforceable standard of 50–200 μg/L [3].

Determination of these species at low detection limits can be carried out by spectroscopic techniques; however this is a time and cost intensive task for products based on aqueous solution (drinking water, beverages, pharmaceutical products), considering that most of the time, under good processing practices, the results fall below the maximum levels allowed. Therefore it would be very convenient to develop a sensor able to alert when one of these metal ions is present above a threshold level without sample conditioning.

Most of these ions are able to form very stable complexes with multidentate chelating ligands capable of capturing ions in ppb concentrations due to their high affinity constants [4–6]. This is an important feature; however the problems of selectivity, generally poor, and of signal transduction still remain. The most popular way of detection is through colorimetric techniques, though sometimes the change in absorbance is not sensitive enough. A partial solution to this problem is the use of chelating agent with fluorescent properties; though it cannot be universally applied. Regarding the selectivity, an ingenious solution was presented by Anzenbacher and co-workers for identification of different metal ions [7] using a set of chelating fluorophores, analyzing the resulting signals by partial component analysis.

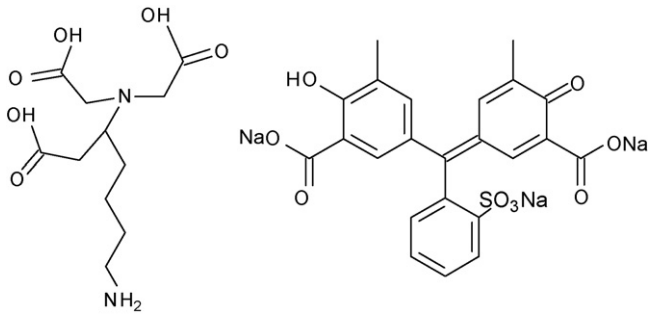
One common characteristic of these positively charged species is the fact that all of them can show electrical effects in the system; however, at low concentrations these changes will not be sufficiently important to produce a sensitive signal. A simple way to enhance their detection is capturing them at an electrode surface, and observing the changes produced in the capacitance or in the electron transfer process of a probe.

In the last years several groups have used the impedimetric response of a surface as transduction method for the detection of species at low concentrations, either by specific recognition, or by pattern recognition methods. In the first case aptamers, antibodies, DNA or recently "covalent virus layer" were use to introduce selectivity [8–10]. Due to the high association constants of these species, it is possible to detect very low concentrations of analytes. In the second case, less selective systems were used to identify groups of compounds rather than individual species [11,12].

In some of these studies a redox couple, i.e. ferri-/ferrocyanide, is used to follow the chemical changes produced on the electrode

* Corresponding author. Tel.: +54 11 45763358; fax: +54 11 45763341.
E-mail address: battagli@qi.fcen.uba.ar (F. Battaglini).

0925-4005/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2010.01.027



Scheme 1. Chemical structures of the chelators used in this work: N,N-bis(carboxymethyl)-L-lysine (dNTA) at left and pyrocatechol violet (PV) at right.

surface. One of the advantages of this method is that it facilitates the analysis of the signal, since the couple is a very well known system. For example, the analyte concentration can be determined by the changes of the electron transfer process at the electrode surface [8]. On the other hand, when probes are not present, the analysis is mainly restricted to capacitance and resistance changes in the system [11,12]. A third approach can be the use of a probe already present at the electrode surface. Changes in the environment can affect the probe's formal redox potential; in impedimetric studies, even changes of few mV can be very important regarding the electron transfer rate at a given potential. This information, plus other electrical changes due to the adsorption of charged species at the electrode surface, may produce a data set useful for distinguishing the nature of the different adsorbed species.

In this work the detection of eight different metal ions (Al^{3+}, Fe^{3+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Ca^{2+} and Ag^{+}) in water is carried out using chelating sensors. The fast reactions between the ions and the chelators concentrate the charged species at the surface of the sensor, and the resulting electric effects are detected by impedance spectroscopy. The sensors are built by simple steps, incorporating two commercially available chelators on the surface: a derivative of nitrilotriacetic acid (dNTA) and pyrocatechol violet (PV) (Scheme 1). The effect of the metal ion on the impedance response of a redox couple bound to the electrode is used to produce a set of data, able to be processed by principal component analysis (PCA), cluster analysis (CA) and multivariate discriminant analysis (MDA). The system allows the identification and detection of these ions at a threshold concentration of 1 μM in different aqueous samples, such as ultrapure water (resistivity 18 MΩ cm), bottled mineral water and a concentrated NaCl solution.

2. Experimental

2.1. Reagents

Aniline, 3-aminophenylacetic acid (3-AFA), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), N,N-bis(carboxymethyl)-L-lysine (dNTA) were provided by Sigma-Aldrich Argentina. Pyrocatechol violet (PV) was from Merck. Metal ion solutions were made from nitrate salts of each cation. All other reagents used were analytical grade. Aniline was distilled prior to use. Ultrapure water (resistivity 18 MΩ cm) was obtained with a Millipore MilliQ water purification system. Bottled mineral water (Villa del Sur, Argentina; lot LB4133), pH 7.8, containing 19 ppm Ca^{2+}, 12 ppm Mg^{2+}, 164 ppm Na^{+}, 10 ppm K^{+}, 450 ppm HCO_3^{-}, 0.7 ppm F^{-}. Concentrated NaCl (27% NaCl) was a gift from Fresenius Medical Care Argentina, the solution is prepared with NaCl and water complaining Pharmacopoeia standards and it is used for the preparation of hemodialysis baths. All the other reagents were analytical grade.

2.2. Sensor construction

2.2.1. dNTA sensor

Scheme 2A shows the disposable eight-graphite electrode array constructed by screen printing in a similar way as previously reported (without silver underlying tracks) [13]. Sequential polymerization of aniline and 3-AFA was carried out by cyclic voltammetry between −0.2 and 0.85 V vs Ag/AgCl at 10 mV s^{-1}. First, the electrodes were immersed in a solution of 90 mM aniline in 1.8 M H_2SO_4 and the potential was simultaneously cycled six times; then, the electrodes were rinsed with water, and immersed in a solution of 10 mM 3-AFA in 1.8 M H_2SO_4 and the potential cycled four times. Finally, the electrodes were rinsed with ultrapure water. The carboxylate groups present in the electrodes were activated with a solution containing 50 mM EDC, 12 mM NHS in MES buffer, pH 5.5 for 30 min to activate the carboxylate groups. After the activation step, the electrodes were rinsed with MES buffer, pH 5.5 and immersed in a 5 mM dNTA in 50 mM PIPES buffer, pH 7.5 for 1 h. Then, the electrodes were rinsed with ultrapure water. Each electrode was divided in two by making an incision with a scalpel, leaving an insulating gap of 40 μm between them. One half was used as working electrode and the other as counter electrode. Finally, the eight sensors are separated and the samples were individually analyzed.The amperometric response of the electrode was stabilized by cycling the electrode at 10 mV s^{-1} between −0.2 and 0.55 V ten times, and then left at 0.2 V vs Ag/AgCl for 200 s.

2.2.2. PV sensor

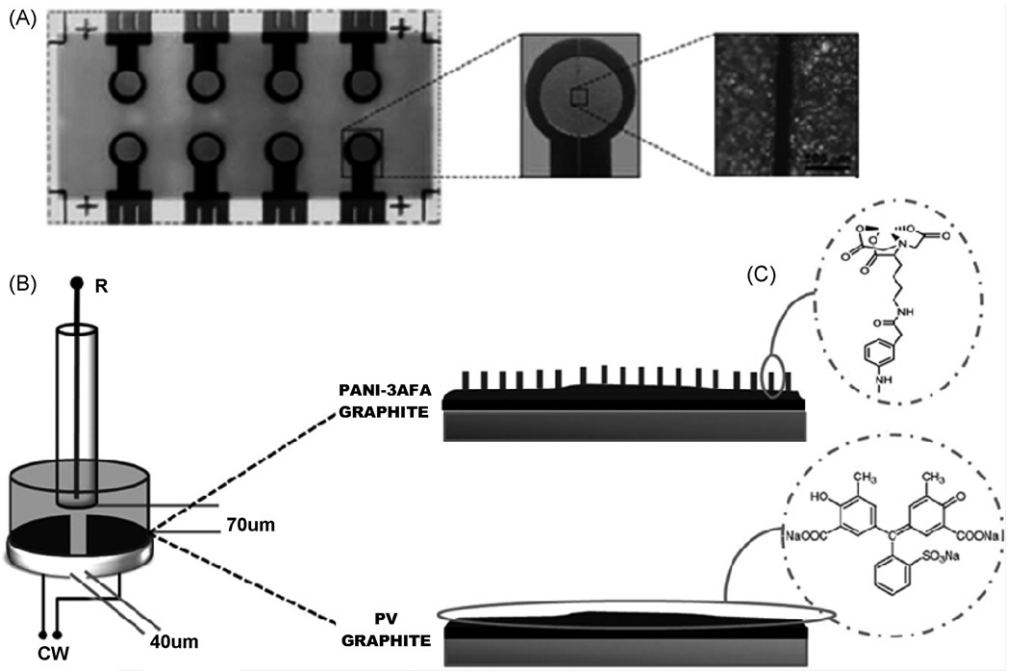
A disposable eight-graphite electrode array was immersed in a 5 mM PV aqueous solution for 90 min, then the electrodes were rinsed with ultrapure water. Each electrode was divided in two by making an incision with a scalpel, leaving an insulating gap of 40 μm between them. One half was used as working electrode and the other as counter electrode. Finally, the eight sensors were separated and the samples are individually analyzed

2.3. Electrochemical impedance spectroscopy (EIS) measurements

EIS measurements were performed using a μAUTOLAB type III impedance analyzer. The cell was made in acrylic, the reference electrode was placed over the working and counter electrodes at a fixed distance of 70 μm. The impedance spectra were recorded within a frequency range of 10^{-1}–10^{4} Hz. The potential was fixed at 0.2 V for the dNTA sensor, and 0.17 V for the PV sensor. The amplitude of the alternating voltage was 10 mV. A total of 51 frequencies were studied with logarithmic distribution in each decade. Each metal ion was investigated in a new modified electrode, where first ultrapure water (or other pure sample) was analyzed and then addition of the respective metal ion to a final concentration ranging from 1 to 250 μM was carried out. Results were analyzed in terms of resistance (Z') and capacitive reactance (Z'').

2.4. Multivariate data analysis

Each measurement was performed using a 2-sensor array, determining Z' and Z'' at a number of 51 frequencies for each sensor. Thus, each measurement was considered as a vector of an N-dimensional vector space with N equal to 204 (2 sensors × 2 variables × 51 frequencies). In addition, considering that four concentrations were tested with three trials for each concentration and for each metal ion, then twelve measurements for each ion were obtained. This renders to a $M \times N$ data matrix composed by M-measurement files and N-coordinate rows, where $M = 12 \times$ number of considered ions to be discriminated (about eight ions plus the solvent). Therefore, a matrix of about $M = 108$ and $N = 204$ was initially used as input for the multivariate analysis. However, the size of the input data matrix



Scheme 2. Schematic representation of the sensor construction. (A) Graphite electrode platform. (B) Experimental cell configuration. (C) Chemical modification of the sensors used in this work.

was significantly reduced later by an appropriated selection of the most representative input data, based on inspection of the loading factors recovered by principal component analysis, which leads to values of N between 18 and 30 depending of the particular case.

2.4.1. Unsupervised methods

Two unsupervised methods, principal component analysis (PCA) and cluster analysis (CA), which are the most commonly methods used for analyzing sensor's array data, were performed [14,15].

In PCA, the projections of each measurement vector into the orthonormal base of the N-dimensional associated to the directions of the maxima data variance were obtained by diagonalizing the correlation matrix. These projections are referred as the so-called principal components. Frequently, the projections into the first two or three directions of the new base of the vector space contains more than 90% of the total data variance, thus investigation of these reduced subset of components improves the ability for grouping data for discrimination purposes providing a substantial dimensional reduction. Hence, the results of PCA are 2D or 3D plots, commonly referred as PCA-maps or score plots, representing the relevant principal components for each measurement performed with the sensor array. The samples are grouped by similarities in these PCA-maps and groups observed by visual inspection.

Cluster analysis (CA) is also an unsupervised method [15]. The main difference respect to PCA is the ability to quantitatively test the performance of the sensor system for the correct clusterization of the data, indicating the power to group the information held in each sample in a number of desired groups. The number of groups, referred as *clusters*, is chosen by the operator following his needs or previous knowledge of the type of samples. In our case the number of chosen clusters was equal to the number of ions plus solvent to be discriminated (for example, nine clusters in the case of ultrapure water solutions).

CA can be implemented using different algorithms In this work the Partition Around Medoids (PAM) algorithm was used, which is based on searching k representative objects among the data set (k = number of target clusters), called *medoids*. The medoids are calculated such as the total distance between all elements and their nearest medoid is minimal. The number of desired classification clusters, to which the input data are going to be assigned after running PAM, must be fixed previously. Each element is then assigned to the cluster corresponding to the nearest medoid. Therefore, PAM indicates by which data input are the clusters composed by; hence the number of correctly grouped samples can be determined.

In addition CA provides also the so-called *silhouette* of each cluster. For each element i (each input data), the silhouette value $sv(i)$ is given by $sv(i) = (a(i) - b(i))/(\max\{a(i), b(i)\})$, where $a(i)$, is the average distance between the element i to all others elements in the cluster, while $b(i)$ is the average distance from i to all others elements of the nearby cluster. If $sv(i)$ is close to 1, the element i is well clusterized, while if $sv(i)$ is close to -1, the element i is badly clusterized. In practice, the optimal clusterization is given by similar values of $sv(i)$ for the different samples within the same cluster, and by values close to 1 of its average, referred as the average silhouette width, ASW. Currently, values of ASW between 0.7 and 1 are considered to indicate a good clusterization [16].

2.4.2. Multivariate discriminant analysis (MDA)

A supervised method, namely multivariate discriminant analysis (MDA) [17,18], was used for classifying samples. MDA uses training data in order to obtain discriminant functions which define decision boundary surfaces between the classes, that is, it finds optimal boundaries which separate classes. Linear or quadratic expressions for the boundary surface function have been used in the present work, referred as LDA and QDA, respectively. The parameters which define those functions are obtained in an optimization process, using the mentioned training data set. For example, LDA finds a linear discriminant function which is a linear combination of the original variables such that the ratio of the between-class scatters and the within-class scatter is maximized, assuming that covariance matrices are equal for all groups.

The amount of training data was always 80% of the total available data and the same percentage was used for selecting the number of data input associated to each class. These data were randomly

selected under the above constrains. This procedure was repeated three times, thus three runs were performed in each one of the analyzed situations.

3. Results

3.1. Sensor construction and impedance response

Since most of the drinking waters have low concentrations of ions, and purified reverse osmosis water is frequently used in the pharmaceutical industry, the electrochemical cell used for this measurements is designed in such a way that the working and the counter electrodes are two graphite parallel strips at a distance of 40 μm, while the reference electrode is placed onto them at 70 μm (Scheme 2B). This arrangement provides a compact system in which the ohmic drop due to the low water conductivity can be handled by a conventional potentiostat and representing a small part the information contained in the signal.

Two types of chelating agents were tested: pyrocatechol violet (PV) and a derivative of nitrilotriacetic acid (dNTA), both presenting different affinities for the metal ions used in this work [4–6]. PV is easily adsorbed on graphite and presents a quasi-reversible redox couple [19] (Scheme 2C). On the other hand, dNTA is not adsorbed and it does not present redox features; however, this derivative, contains a primary amino group that allows the covalent attachment to carboxylic groups (Scheme 2C). The graphite electrode was modified by electropolymerization of a thin layer of polyaniline (PANI) and; then, 3-aminophenylacetic acid (3-AFA) was electropolymerized onto the formed polymer, as reported for a similar system constructed with aniline and a sulfonic derivative of aniline [20]. In this way, not only acetic groups are attached on the surface for further modification, but also the PANI remains electroactive at neutral pH generating a redox probe that can provide information to the system (Fig. 1).

3.1.1. Ultrapure water analysis

As described in the experimental section, graphite electrodes were built by screen printing techniques in plates containing eight electrodes, and latterly modified simultaneously to obtain a better reproducibility. Fig. 2 shows the Nyquist plot, resistance (Z') vs capacitive reactance ($Z'' = 1/\omega C$), for a PV modified electrode exposed to ultrapure water. The bars on each point represent the standard deviation obtained for eight different electrodes; for example the average values for the resistance and the capacitive

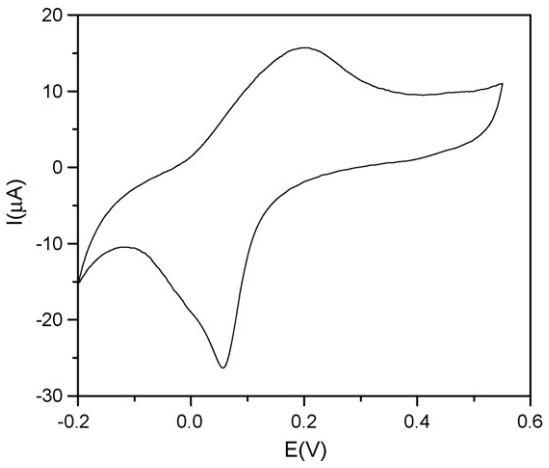


Fig. 1. Cyclic voltammogram of a graphite electrode modified by consecutive electropolymerization of aniline and 3-AFA in 50 mM Tris buffer, pH 7.0. Sweep rate 10 mV s^{-1}.

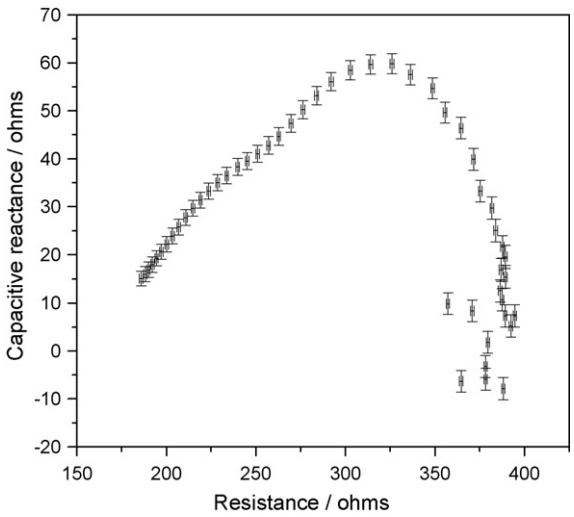


Fig. 2. Nyquist plot corresponding to the average of eight electrodes modified with PV in presence of ultrapure water. The bars indicate the standard deviations in resistance and capacitive reactance for each measurement. Applied potential 0.17 V vs Ag/AgCl, alternating potential amplitude 10 mV.

reactance at 10 Hz are 364 ± 2 and 46 ± 1 ohms ($n = 8$), respectively. The fabrication reproducibility permits correlate results, exposing the modified electrodes to different metal ion solutions, without the need of relating them to a common reference; a similar behavior is observed for the dNTA sensors

Another important characteristic of the system is that, due to the fast reaction rate of the metal ion with the chelator, measurement can be carried out immediately after placement of the sample in contact with the electrode, without any further manipulation, such as rinsing steps or probe additions.

51 frequencies were tested from 0.1 to 10,000 Hz, giving current and phase shift responses for each of them. This information can be represented as resistance (Z') and capacitive reactance (Z''), therefore the sample produces 102 responses in each sensor. The data obtained at some selected frequencies are plotted in Fig. 3. In the upper row are represented the resistance (left) and the capacitive reactance (right) for the PV sensor. The resistance values for each metal ion do not show important changes through the frequencies, however among the samples some features can be easily noted, and they are related to their chemical characteristics. Three main groups can be established: a first group (Cu^{2+} and Hg^{2+}) with a low resistance, it has to be mentioned that these ions undergo a redox process at the working potential; a second group (Fe^{3+} and Al^{3+}) with an intermediate resistance, presenting the highest affinity constants for the chelator; and finally a third group, corresponding to the ions that are not complexed or present a low affinity to PV [4]. To the right of the graph, the plot of the capacitive reactance shows a more diverse pattern throughout the frequencies, also allowing the classification of the metal ions mainly in the same three groups, as before.

For the dNTA sensor (lower row in the graph), the changes in resistance throughout the frequency range are small; yet, an important change in capacitance is observed. In this case the values for the capacitive reactance of water are not plotted since they range from 28 to 31 ohms, against a maximum value of 3 when a metal ion is present. It is important to note that these values represent the inverse of the capacitance; considering that this sensor is formed by a coating containing weak acid and base moieties, when a metal ion is incorporated an important change in the coating charge occurs, impacting on the capacitance.

Even though the qualitative interpretation of the results addressed above is possible, a detailed analysis cannot be done;

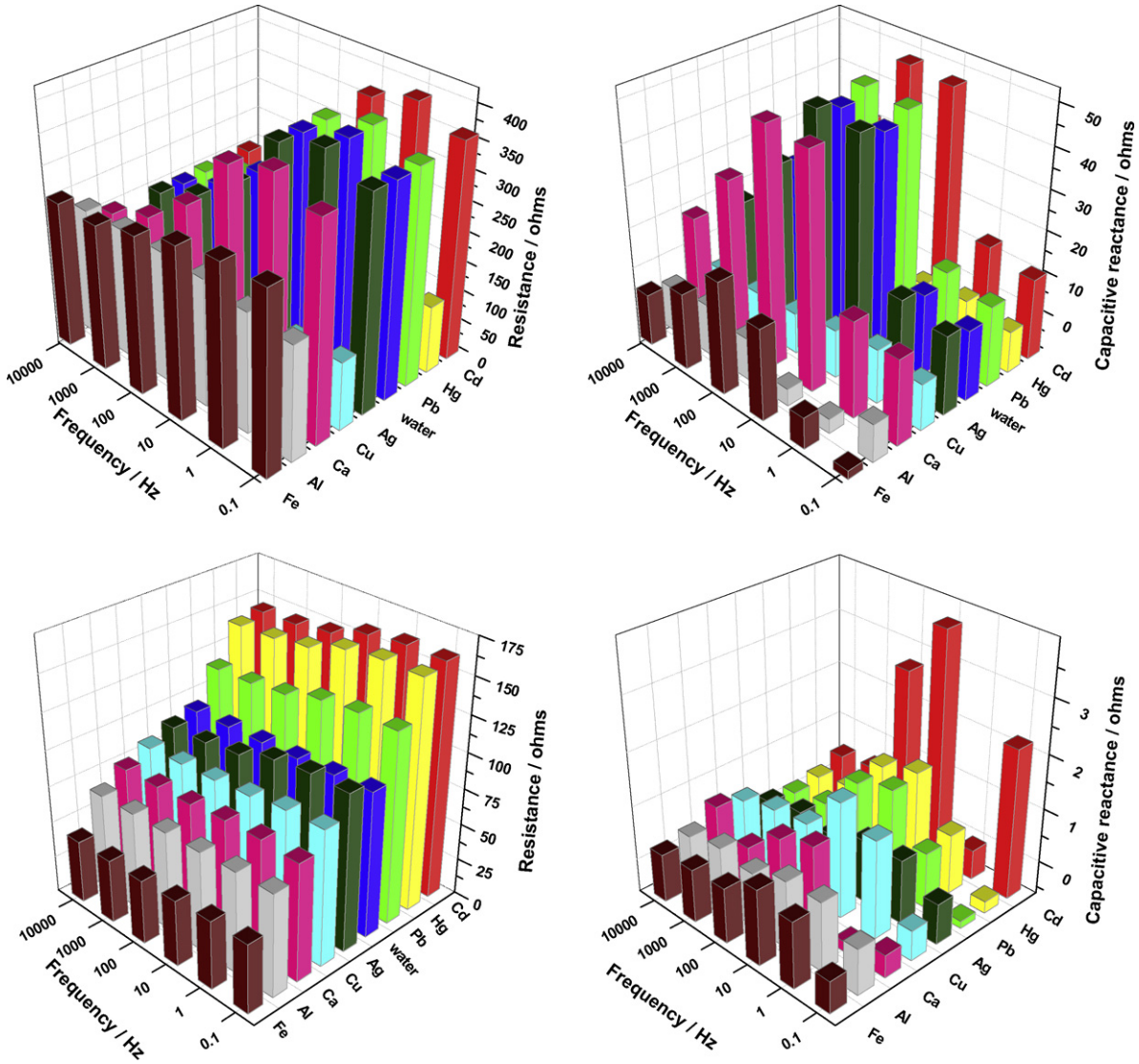


Fig. 3. Sensor responses of ultrapure water and 12 μM metal ion solutions in ultrapure water. Resistance (left) and capacitive reactance (right) at selected frequencies. PV sensors (top), dNTA sensors (bottom).

since the experiments are carried out in conditions in which the behavior of the different cations cannot be described by means of a simple model. In this case our focus is placed in using the wealth of information for searching differences that may allow us detecting the different ions above low threshold concentrations. Therefore, multivariate data analysis was performed (PCA, CA and MDA) in order to explore the ability of the present methodology for discriminating solutions of different ions, that is, on the basis of its chemical identity.

The first approach to determine the metal ions was to analyze the 204 responses for each sample by PCA at four different concentrations, from 1 to 250 μM by triplicate. The first principal component contains the highest degree of variance and other components follow in the order of decreasing variance, in this way the pattern generated by the sensor array is reduced to a single score and plotted in the new space generated using only two or three principal components. This representation (score plot) is shown in Fig. 4. Here, the PCA score plot utilizes the first three principal components which accounts for 94% of the total data variance and it shows an unambiguous clustering. Since PCA is an unsupervised method, namely, the formed groups are defined by the similarity of the samples, it can be used to test the ability of the applied method to discriminate the different cation solutions; as it can observed from the figure, nine clusters can be easily recognized by visual inspection in the PCA map. Each cluster is composed only by one type of sample, that is, ultrapure water or each one of the cation aqueous solutions. Taking only the two first principal components still can be observed a clear grouping of samples with a good discrimination among the different pollutants (Fig. 4, below). It is worth mentioning the important difference between ultrapure water and the other samples, while the distribution of the ions in the plot maintains a relation with the details described before. For example, Hg^{2+} and Cu^{2+} present a position strikingly different respect to the others; while Fe^{3+} and Al^{3+} are close.

Cluster analysis (CA) was performed in order to quantitatively confirm the excellent grouping obtained by visual inspection in PCA. In fact, a 100% of correctly clusterized samples were obtained when CA was performed for a number of 107 samples, that is, all the samples with the same composition were assigned within a same cluster with no mistakes. The average silhouette width, ASW, was 0.86, confirming the goodness of the clusterization.

Finally, a supervised method, MDA, was also performed. Considering that the method is thought as an alarm for metal ion contamination in the production of pharmaceuticals or beverages

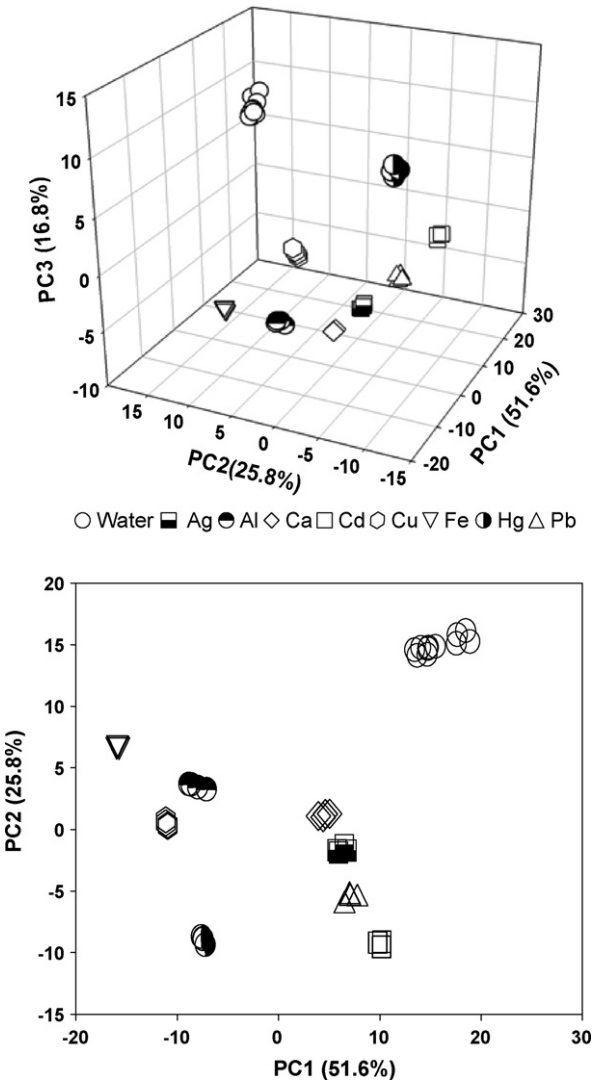


Fig. 4. 3D (top) and 2D (bottom) PCA plots for ultrapure water and the same sample contaminated with different cations. Ion concentrations range between 1 and 250 μM.

in situations where it is known the possible presence of certain impurities, the ability of the sensor system to classify samples of the considered cations was explored. This is carried out by using supervised multivariate data analysis, as *multivariate discriminant analysis (*MDA). In other words, supervised multivariate methods can be used to train the sensor system to recognize relevant contaminants. *MDA was used for classifying the samples.* 86 samples were randomly chosen to train the system and 21 as testing samples. This process was randomly repeated three times and in all the cases 100% of correct classification of the testing samples was obtained.

Since the system shows an excellent performance, it arise the question if a lesser number of data input can be used to obtain the same result, and save time in the analytical procedure. Two criteria were taken, one statistical, based on the analysis of the most relevant frequencies used in PCA, and the other by visual inspection of Z' and Z'' obtained at different frequencies (Fig. 3). The statistical criterion, based on the analysis of the so-called loading factor obtained in PCA, shows that both sensors are needed; in each of them, Z' and Z'' are used in the analysis. Finally, the statistical analysis shows that low frequencies (among 0.1 and 32 Hz) contribute to produce a good discrimination of the different type of samples. Using 18 variables a 97% variance is obtained within the three first PCs. For the cluster analysis, if the samples are divided in nine clusters, the system confuses some of the ultrapure water samples with silver ion solutions. This can be corrected using the supervised method (MDA) where a success of 100% is obtained.

Analyzing Fig. 3, it can be observed that the system at low frequencies shows important differences among the ion solutions. Therefore, seven frequencies were analyzed between 0.1 and 40 Hz, using the two sensors. This means a total of 28 variables were analyzed. Cluster analysis is improved with 100% of success, meaning that with the use of only 28 of the 204 variables it is possible to obtain the same result. MDA also presents 100% of correct classification of the testing samples.

3.1.2. Analysis of other type of samples

The presence of metal pollutants in aqueous solutions is an important concern in beverages and pharmaceutical products. As examples of the applicability of this method to the detection of eventual contaminants, concentrated NaCl solutions of pharmacopoeia quality, and bottled mineral water were analyzed.

In concentrated NaCl solutions, the most important changes respect to the previous sample are the high ion concentration, the ability of chloride ions to form complexes with metal ions and the presence of sodium. The pure solution presents important differ-

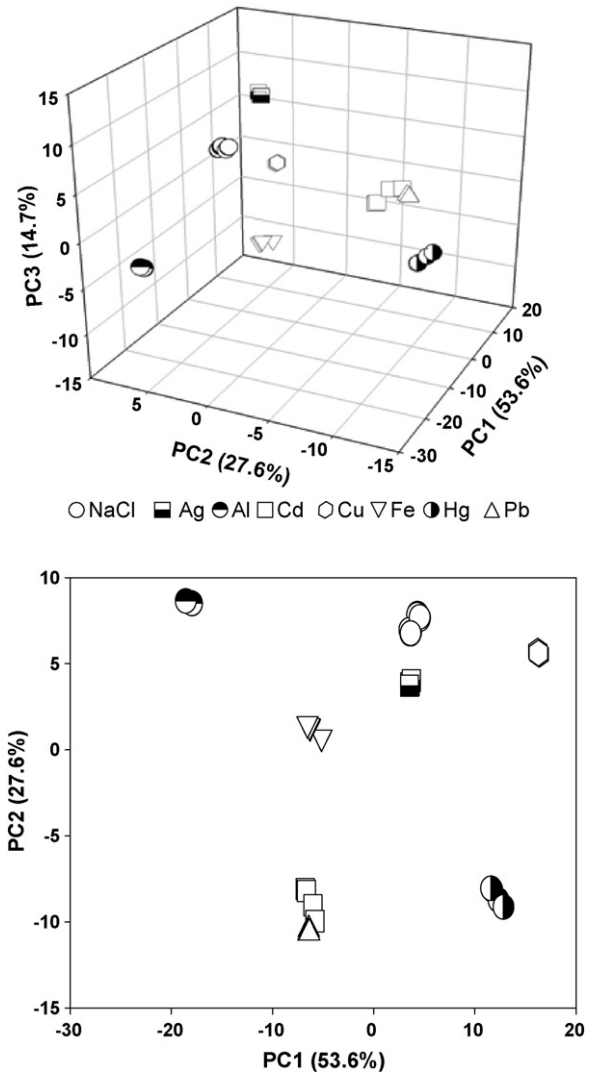


Fig. 5. 3D (top) and 2D (bottom) PCA plots for concentrated NaCl solution and the same sample contaminated with different cations. Ion concentrations range between 1 and 250 μM.

ences respect to ultrapure water; however the detection of the metal ions is possible due the high sequestration capabilities of the chelators. Fig. 5 shows the principal component analysis of the NaCl solution contaminated with the different metal ions; it can be observed that the three first PCs takes into account 96% of the variance (top figure), while the 2D takes into account 81% of the variance (bottom figure). In both graphs well defined regions are obtained; compared with the ultrapure water, the distribution of the different cluster around the pure sample is different, and the system tends to merge Cd^{2+} and Pb^{2+} samples. If CA is applied to clusterize the samples, it shows that the analysis integrates Pb^{2+} and Cd^{2+} in the same group, while Hg^{2+} is divided in two groups. Finally, MDA shows that with an appropriate training, similar to the one carried out in ultrapure water, the system can differentiate all the samples (100% success).

The statistical analysis shows that the most relevant variables in PCA are among 400 and 10 KHz. Taken the six most relevant for the three first principal components, a 97% variance is obtained. In Fig. 6 can be observed the plot of the results, with a better discrimination for Cd and Pb than the one showed in Fig. 5. Also, using only these 18 variables, cluster analysis is able to discriminate Pb from Cd, assigning to each ion its own cluster with an ASW of 0.84, showing that sometimes an excess of information can be redundant given misleading information.

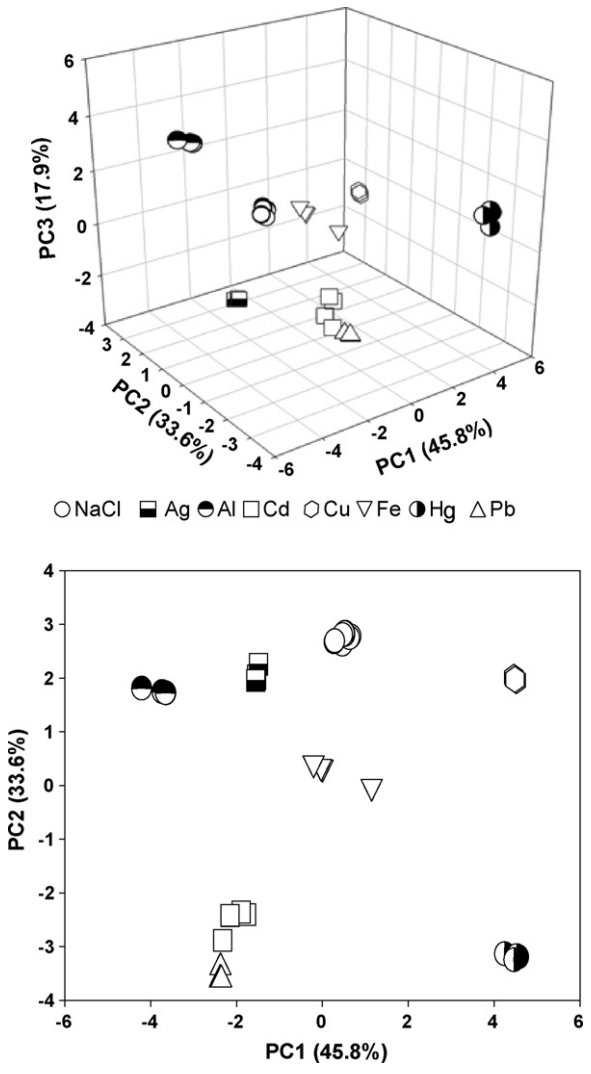


Fig. 6. 3D (top) and 2D (bottom) PCA plot for the same samples than in Fig. 6. In this case only 18 variables were taken into account for the analysis.

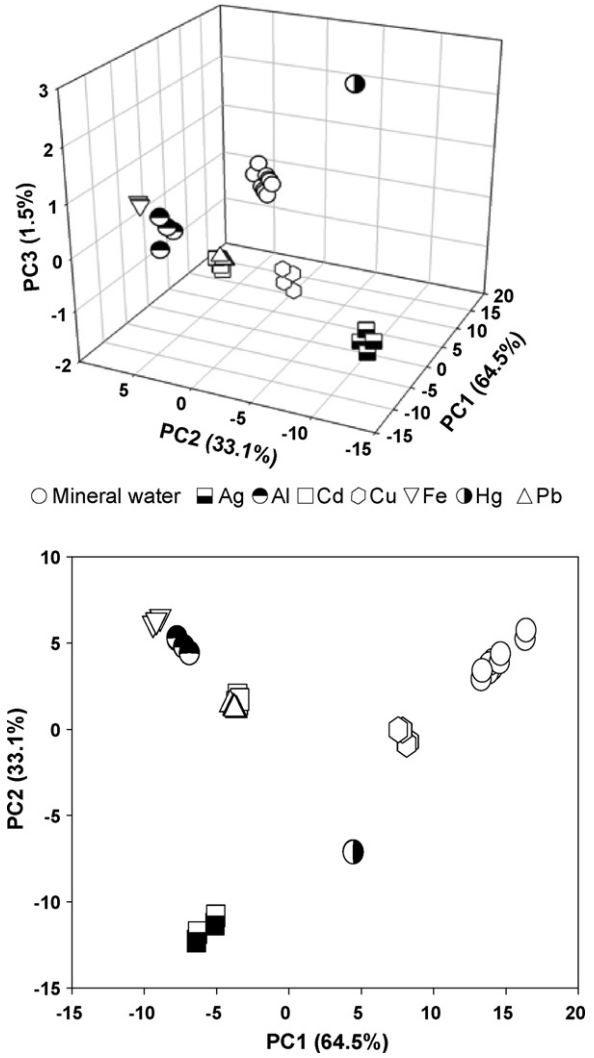


Fig. 7. 3D (top) and 2D (bottom) PCA plots for bottled mineral water and the same sample contaminated different cations. Ion concentrations range between 1 and 250 μM.

The third sample studied in this work was bottled mineral water, where Ca and Mg are at concentration around 500 μM. The chelating capabilities of the agents used toward Ca and Mg may have an important impact, therefore PCA was carried out for each sensor independently. The sensor containing PV can only produce two big groups, one containing the pure mineral water samples, and the other containing all the contaminated samples. In this case, even though calcium is unable to form complexes with PV, magnesium undergoes complexation [4,21]. On the contrary, the sensor modified with dNTA can discriminate practically all the contaminants, showing only a superposition between Pb from Cd (Fig. 7). In this case the complexation constant for Ca and Mg with NTA are at least three orders of magnitude smaller than the ones for the other metal ions [5,6]. When CA is carried out, Pb and Cd are placed in the same group. In this case the use of MDA is the great importance since with a proper training (78 training samples) the system is able to identify 20 testing samples, including the correct identification of Pb and Cd (100% of right classification).

For this type of samples the six most relevant variables in the three first principal components belongs to high frequencies. In this case, its use in PCA and CA does not improve the previous results. However, MDA is able to classify correctly all the samples (100% success).

4. Discussion and conclusions

This work shows that the trace detection and discrimination of different metal ions can be carried out by analyzing the impedance response of modified electrodes with chelators; the combination of strong sequestering agents with a sensitive technique to the adsorption of charged species on a conducting surface produce a device able to detect metal ions at a threshold concentration of 1 μM in the three different samples.

The method allows the detection of metal ions in aqueous samples without the need of sample conditioning, rinsing steps or the addition of probes. The system is easy to construct and unexpensive. The fact that the sensors can be constructed and modified in batches guaranties the reproducible of the assay without to take references for the data analysis.

Due to the distance among the electrodes, the sensor system can be work in environments of different conductivities, one of very low conductivity (ultrapure water) and the other of high conductivity (concentrated NaCl), and in both of them easily distinguish the different types of pollutants. In the case of aluminum, a concentration of 27 ppb can be detected, below the limit given by EPA for drinking water. The performance of the system can be also improved by an appropriate analysis of the signals at different frequencies; sometimes is possible to choose the right frequencies at a first glance of the system response; while, if the data look confusing, the use of statistical algorithms allow choosing the most significant information.

The third type of sample analyzed was mineral water; in this case the sample contains Ca and Mg ions that can be complexed by the chelating agents. The concentration of Ca and Mg were around 500 μM, while the contaminants range from 1 to 250 μM. In this case the analysis was not straight forward and the sensor containing PV was unable to discriminate the different pollutants, while the use of the one containing dNTA practically allowed the whole discrimination.

The existence of a myriad of chelating agents allows developing the most convenient taste sensor for each application. To achieve successfully this goal, it will be necessary to take into account the basic chemical features of the sample components and the expected pollutants to choose the appropriate chelating agents. The method opens the opportunity to develop simple systems to guarantee the quality of water in the most diverse areas.

Acknowledgments

Universidad de Buenos Aires and ANPCyT (PICT 00575) are acknowledged for financial support. FB and RMN are research staff members of CONICET. JYH and SDR are recipients of CONICET fellowships.

References

[1] I. Shcherbatykh, D.O. Carpenter, The role of metals in the etiology of Alzheimer's disease, J. Alzheimer's Dis. 11 (2007) 191–205.
[2] T.P. Flaten, Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water, Brain Res. Bull. 55 (2001) 187–196.
[3] Secondary Drinking Water Regulations: Guidance for Nuisance Chemicals EPA 810/K-92-001, http://www.epa.gov/safewater/consumer/2ndstandards.html, July 1992.
[4] K. Ueno, T. Imamura, K.L. Cheng (Eds.), Handbook of Organic Analytical Reagents, 2nd ed., CRC, Boca Raton, Florida, 1992.
[5] S. Chaberek, A.E. Martell, Organic Sequestering Agents, Wiley, New York, 1959.
[6] I.M. Kolthoff, E.B. Sandell, E.J. Meehan, S. Bukenstein, Quantitative Chemical Analysis, 4th ed., The Macmillan Company, Toronto, Canada, 1969.
[7] M.A. Palacios, Z. Wang, V.A. Montes, G.V. Zyryanov, P. Anzenbacher Jr., Rational design of a minimal size sensor array for metal ion detection, J. Am. Chem. Soc. 130 (2008) 10307–10314.
[8] M.C. Rodriguez, A.-N. Kawde, J. Wang, Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of the surface charge, Chem. Commun. (2005) 4267–4269.
[9] E. Katz, I. Willner, Probing biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy: routes to impedimetric immunosensors, DNA-sensors, and enzyme biosensors, Electroanalysis 15 (2003) 913–947.
[10] L.-M.C Yang, J.E. Diaz, T.M. McIntire, G.A. Weiss, R.M. Penner, Direct electrical transduction of antibody binding to a covalent virus layer using electrochemical impedance, Anal. Chem. 80 (2008) 5695–5705.
[11] G. Pioggia, F. Di Francesco, A. Marchetti, M. Ferro, R. Leardi, A. Ahluwalia, A composite sensor array impedentiometric electronic tongue: Part I. Characterization, Biosens. Bioelectron. 22 (2007) 2618–2623.
[12] M. Ferreira, A. Riul Jr., K. Wohnrath, F.J. Fonseca, O.N. Oliveira Jr., L.H.C. Mattoso, High-performance taste sensor made from Langmuir–Blodgett films of conducting polymers and a ruthenium complex, Anal. Chem. 75 (2003) 953–955.
[13] G. Priano, G. Gonzalez, M. Günther, F. Battaglini, Disposable gold electrode array for simultaneous electrochemical studies, Electroanalysis 20 (2008) 91–97.
[14] R.A. Johnson, D.W. Wichern, Applied Multivariate Statistical Analysis, Prentice Hall, New Jersey, 2002.
[15] A. Struyf, M. Hubert, P.J. Rousseeuw, Integrating robust clustering techniques in S-PLUS, Comput. Stat. Data Anal. 26 (1997) 17–37.
[16] L. Kaufman, P.J. Rousseeuw, Statistical data analysis based on the L_1 norm, in: Y. Dodge (Ed.), Clustering by Means of Medoids, North-Holland, Amsterdam, 1987, pp. 405–416.
[17] S.M. Scott, D. James, Z. Ali, Data analysis for electronic nose systems, Microchim. Acta 156 (2006) 183–207.
[18] L.A. Berrueta, R.M. Alonso-Salces, K. Héberger, Supervised pattern recognition in food analysis, J. Chromatogr. A 1158 (2007) 196–214.
[19] C.-X. Cai, L.-H. Yin, K.-H. Xue, Electrocatalysis of NADH oxidation at a glassy carbon electrode modified with pyrocatechol sulfonephthalein, J. Mol. Catal. A: Chem. 152 (2000) 179–186.
[20] J. Yánez-Heras, A.F. Forte-Giacobone, F. Battaglini, Ascorbate amperometric determination using conducting copolymers from aniline and N-(3-propane sulfonic acid)aniline, Talanta 71 (2007) 1684–1688.
[21] R. Kocjan, M. Garbacka, Additional purification of alkali or alkaline earth salts by using silica gel modified with pyrocatechol violet as a sorbent, Talanta 41 (1994) 131–133.

Biographies

Jorge Yánez Heras is a graduate student at Universidad de Buenos Aires. He received his BcS degree in chemistry from Universidad Central de Venezuela in 2004.

Silvio D. Rodríguez is a graduate student at Universidad de Buenos Aires. He received his BcS degree in Food Technology from Universidad Nacional de Quilmes in 2006.

R. Martín Negri is an assistant professor at Universidad de Buenos Aires and research staff of the Argentina Research Council (CONICET). He received his PhD from Universidad de Buenos Aires in 1991. His research interests are chemical sensors and sensor's arrays (electronic noses-tongues), multivariate data analysis and material science.

Fernando Battaglini is an associate professor at Universidad de Buenos Aires and research staff of the Argentina Research Council (CONICET). He received his PhD from Universidad de Buenos Aires in 1991. His research interests are biosensors, electrochemistry and materials science.