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A composite sensor array impedentiometric electronic tongue Part II. Discrimination of basic tastes

G. Pioggia[a,*], F. Di Francesco[b], A. Marchetti[a], M. Ferro[a], R. Leardi[c], A. Ahluwalia[a]

[a] Interdepartmental Research Center "E. Piaggio", Faculty of Engineering, University of Pisa, Italy
[b] Department of Chemistry and Industrial Chemistry, University of Pisa, Italy
[c] DICTFA, University of Genoa, Italy

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Abstract

An impedentiometric electronic tongue based on the combination of a composite sensor array and chemometric techniques aimed at the discrimination of soluble compounds able to elicit different gustative perceptions is presented. A composite array consisting of chemo-sensitive layers based on carbon nanotubes or carbon black dispersed in polymeric matrices and doped polythiophenes was used. The electrical impedance of the sensor array was measured at a frequency of 150 Hz by means of an impedance meter. The experimental set-up was designed in order to allow the automatic selection of a test solution and dipping of the sensor array following a dedicated measurement protocol. Measurements were carried out on 15 different solutions eliciting 5 different tastes (sodium chloride, citric acid, glucose, glutamic acid and sodium dehydrocholate for salty, sour, sweet, umami and bitter, respectively) at 3 concentration levels comprising the human perceptive range. In order to avoid over-fitting, more than 100 repetitions for each sample were carried in a 4-month period. Principal component analysis (PCA) was used to detect and remove outliers. Classification was performed by linear discriminant analysis (LDA). A fairly good degree of discrimination was obtained.
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Keywords: Composite sensor array; Impedentiometric electronic tongue; Taste discrimination; PCA; LDA

1. Introduction

The main interest in the development of the so-called electronic noses (e-nose) and tongues (e-tongue) (Lavigne et al., 1998; Pearce et al., 2002) comes from the request of low cost, high throughput, versatile pseudo-analytical instruments capable of replacing expensive and inefficient human panels in the assessment of products (food, beverage, packaging, etc.) (Ampuero and Bosset, 2003), as well as to lower the cost of selected urine and blood tests or to serve as screening for medical diagnosis (Logrieco et al., 2005; Machado et al., 2005; Shykhon et al., 2004), and to monitor the quality of the environment (Persaud et al., 2005). So far, much more attention has been paid to the development of e-noses compared to e-tongues. From a biological point of view, important synergies exist among senses that concur to determine perceptions. Behavioural reactions to concordant multisensory cues exhibit lower thresholds than their unisensory counterparts, while cross-modal cues that are significantly discordant can have the opposite effect and depress responses (Calvert, 2000). For example, the pairing of a sub-threshold odour and a sub-threshold taste results in a decrease of the odour threshold (Dalton et al., 2000). From an instrumental point of view, it would be interesting to combine an e-nose and an e-tongue to obtain complementary information on the same sample by means of a hybrid array (Wide et al., 1998). Since most e-nose sensors are based on variations in electrical resistance when exposed to odours, the impedance measurement represents the easiest way to use the same electronic front-end for both gas and liquid sensors. This would mean, in perspective, the possibility to develop really low cost instruments, which is a must to make this technology exit from laboratories. But experience has taught that great attention has to be paid, when dealing with e-noses and e-tongue to avoid inappropriate generalization of results which are only valid in a limited region of the experimental domain. Goodner et al. (2001) illustrated the risk of data over-fitting, which can lead

* Corresponding author. Tel.: +39 050 2217056; fax: +39 050 2217051.
E-mail address: giovanni.pioggia@ing.unipi.it (G. Pioggia).

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to counterfeit classifications. According to these authors the ratio between samples and variables should be greater than six in order to obtain reliable results. Moreover, in order to guarantee measurement accuracy, reliability and repeatability, aside from considering the volatile nature of the substances, the sampling system must control and optimise all factors capable of influencing the generation of sensor transduction signals, while the electronic apparatus must be fast and accurate.

In this paper we address the development of an impedentiometric electronic tongue based on the combination of a composite sensor array and chemometric techniques, i.e. principal component analysis (PCA) and linear discriminant analysis (LDA) (Brereton, 2003), in order to verify its ability in distinguishing among soluble compounds. Five different sensors were fabricated and characterized in Part I (Pioggia et al., 2007). They comprise three different recognition mechanisms: a carbon nanotube (CNTs) loaded hydrogel, two commercial polymers loaded with carbon black and two conducting polymers. The sensor responses depend on a complex interplay between solution permeation in the matrix, electromechanical response times and ion affinity/mobility. Each sensor type has a distinct recognition mechanism, with a different dynamic response, and it is this varied array which contributes to the overall discrimination power of the e-tongue. The system was tested with five compounds with different chemical characteristics (a carbohydrate, two salts, a weak organic acid and an amino acid) able to elicit different kinds of gustative perceptions (glucose, sodium dehydrocholate, sodium chloride, citric acid and glutamic acid) representing the five classic tastes. Over 100 measurements were carried out over a 4-month period at three concentration levels of each solution comprising the human sensitivity range to evaluate the system discrimination capability.

2. Experimental methods

The current research in electronic tongues is based mainly on electrochemical measurements, with the main focus being on the development of novel sensors, such as membranes and electrode coatings. Indeed, the use of impedance measurement represents a novel approach for the realisation of an electronic tongue. This approach is justified by the affinity with the measurement of resistance variations which is the most commonly used method for electronic noses. Our objective is to merge both olfactive and gustative sensing into a human-like taste and smell perception system; the impedance sensing method simplifies the integration process. In particular, the use of three different sensing layers was investigated. The first sensing layer consisted of a hydrophilic hydrogel consisted of a blend of poly(vinylalcohol) (PVA) and polyallylamine (PAA) loaded with CNTs. The second sensing layer was realised from a matrix of polylactic acid (PLA) loaded with carbon black and it is based on the method described by Lonergan et al. (1996). In the third case, a sensing layer consisting of poly(alkoxy-bithiophenes) previously used to detect organic vapours (Gallazzi et al., 2003), was adopted. Preparation and characterization of the sensing layers is reported in Part I (Pioggia et al., 2007). The composite sensor array capability of discriminating different solutions in a concentration range typical of human foods is reported here.

2.1. Measurement protocol

The electrical impedance of the sensor array was monitored at a frequency of 150 Hz. The data acquisition system is reported in Part I Pioggia et al. (2007). The following measurement protocol was adopted:

(a) sensors in air, start of data acquisition;
(b) sensors dipped in distilled water;
(c) sensors in air;
(d) sensors in solution 1;
(e) new cycles (a)–(d) for the other solutions;
(f) sensors in air, stop acquisition.

At the end of this run of measurements, 36 values of both modulus and phase were acquired for each solution relevant to air (16 data), distilled water (10 data) and solution (10 data). The classification capability of the device was tested on 15 solutions of the 5 compounds at 3 concentration levels (Table 1) chosen so as to cover the human range of sensitivities.

To avoid the over-fitting of the classification process (Goodner et al., 2001), a large measurement campaign was adopted. A 106 repetitions for each solution (a total of 1590 measurements) were organised in six different campaigns carried out over 4 months and measured following a random order (Table 2). This rigorous testing also demonstrated the reproducibility of the sensor responses and their stability over time.

2.2. Data analysis

The data were collected in a database (MySQL), then suitable queries allowed the extraction of the steady state impedances of sensors which were analysed by using multivariate techniques.

Table 1
Taste solutions, concentrations and labels

Taste	Compound	Concentration (M)					
		Low		Middle		High	
Acid	Citric acid	0.005	CAL	0.05	CAM	0.5	CAH
Bitter	Sodium dehydrocholate	0.0001	SDL	0.001	SDM	0.01	SDH
Sweet	Glucose	0.001	GL	0.01	GM	0.1	GH
Salty	Sodium chloride	0.001	SCL	0.01	SCM	0.1	SCH
Umami	Glutamic acid	0.001	GAL	0.01	GAM	0.06	GAH

Table 2
Experimental sessions

Session		Number of repetitions	Total number of measurements
Number	Starting day		
I	1	30	450
II	15	30	450
III	30	30	450
IV	60	6	90
V	91	6	90
VI	120	5	75

Before applying any classification technique, the identification and the removal of outliers (samples significantly different from analogues belonging to the same population) was accomplished. As samples of each category were obtained in our case by replicates of the same measurement procedure, a multivariate normal distribution around an ideal category representative might be expected, deviations from that point being due to experimental errors. Large deviations may be generated by random errors either in the sample preparation and measurement or in the data acquisition and treatment, so that it is not possible to consider the resulting object a typical sample of that category. Principal component analysis (PCA) (Brereton, 2003) was used to display data and detect outliers, by using the Q and T^2 diagnostics. Samples identified as outliers for at least one of these statistics with $p < 0.001$ (more than three standard deviations from the mean value of any category) were removed.

Classification was performed by linear discriminant analysis (LDA) (Brereton, 2003). Two different schemes of cross-validation were applied. The first one was the standard Venetian blind with five deletion groups, in which the first deletion group is formed by samples 1, 6, 11, ..., the second deletion group by samples 2, 7, 12, ..., and so on. This procedure does not test the robustness of the model versus the sensor drift, since samples measured the same day are spread throughout all the deletion groups. To overcome the problem and get results as close as possible to a real prediction, a second approach (leave-1-day-out) was applied. Six deletion groups, each composed of all the samples measured on the same day, were created. In order to compensate for the different sample size in the different days, the predictive ability of each category was computed as the average of the predictive abilities of the same categories in the 6 days.

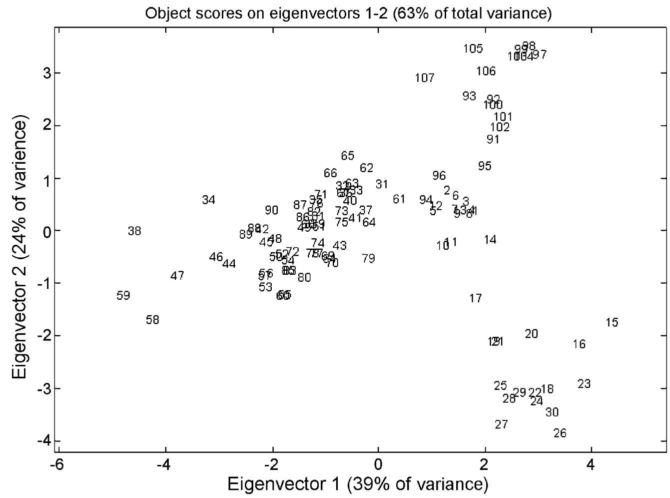

Fig. 1. PCA score plot of the SCH measurements.

3. Results and discussion

For each of the 15 categories (5 tastes and 3 different concentrations), a dataset containing the extracted steady-state values of the impedance magnitude and phase, was obtained and a PCA was performed to detect and remove outliers according to the procedure previously outlined. As an example, Fig. 1 shows the score plot of the SCH measurements (see Table 1); samples obtained in the first measurement session (samples 1–30) are clearly different, and therefore they are removed.

The distribution of the samples according to the category and the measurement session after the elimination of the outliers is reported in Table 3.

Table 3
Measurement session after the elimination of the outliers

		I	II	III	IV	V	VI	Total
1	Citric acid low (CAL)	28	30	30	6	1	5	100
2	Citric acid middle (CAM)	29	30	30	6	6	5	106
3	Citric acid high (CAH)	22	30	30	6	6	5	99
4	Sodium dehydrocholate low (SDL)	29	30	30	6	6	4	105
5	Sodium dehydrocholate middle (SDM)	27	30	26	6	6	5	100
6	Sodium dehydrocholate high (SDH)	30	30	30	6	6	5	107
7	Glucose low (GL)	30	30	30	5	0	0	95
30	Glucose middle (GM)	0	30	30	6	9	0	75
9	Glucose high (GH)	30	30	30	6	6	5	107
10	Sodium chloride low (SCL)	28	30	30	6	6	5	105
11	Sodium chloride middle (SCM)	30	30	30	6	6	5	107
12	Sodium chloride high (SCH)	0	30	30	6	6	5	77
13	Glutamic acid low (GAL)	30	30	24	6	6	5	101
14	Glutamic acid middle (GAM)	28	30	29	6	6	3	102
15	Glutamic acid high (GAH)	30	30	30	6	6	0	102
Number of retained samples		371	450	439	89	82	57	
Retained samples (%)		82.4	100	97.6	98.9	91.1	76.0	

Table 4
Confusion matrix after the Venetian blind cross-validation

	CAL	CAM	CAH	SDL	SDM	SDH	GL	GM	GH	SCL	SCM	SCH	GAL	GAM	GAH	Correct predictions (%)
CAL	73	0	0	0	0	20	0	0	0	0	0	0	0	0	7	73.0
CAM	0	101	0	0	0	0	0	0	0	0	5	0	0	0	0	95.3
CAH	0	1	98	0	0	0	0	0	0	0	0	0	0	0	0	99.0
SDL	0	0	0	57	4	0	36	4	4	0	0	0	0	0	0	54.3
SDM	0	0	0	0	60	0	0	12	15	1	0	0	12	0	0	60.0
SDH	39	0	0	0	0	56	0	0	0	0	0	0	0	0	12	52.3
GL	0	0	0	25	0	0	67	1	2	0	0	0	0	0	0	70.5
GM	0	0	0	20	5	0	4	12	13	1	0	0	15	3	2	16.0
GH	0	0	0	9	10	0	10	14	57	2	0	0	5	0	0	53.3
SCL	0	0	0	0	0	0	0	0	0	87	0	0	0	16	2	82.9
SCM	1	0	0	0	0	2	0	0	0	0	104	0	0	0	0	97.2
SCH	0	0	0	0	0	0	0	0	0	0	0	77	0	0	0	100.0
GAL	0	0	0	0	26	0	0	9	1	1	0	0	62	2	0	61.4
GAM	0	0	0	0	0	0	0	0	0	21	0	0	0	80	1	78.4
GAH	9	0	0	0	0	4	0	0	0	0	0	0	0	0	89	87.3
Mean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	72.1

Several samples of the first session had to be removed (e.g. all the GM and SCH), as well samples of the last session, which showed a high percentage of outliers. In the end, 1488 samples out of 1605 were retained (92.7%).

As regards LDA, at first, the Venetian blind cross-validation approach was applied, which can produce overoptimistic results in case of time drift of data. The confusion matrix (Table 4), in which the *ij*th element represents the number of samples from class *i* that were classified as class *j*, allows a complete evaluation of the performance of the model. Correct predictions are represented by the elements *ii* on the principal diagonal; the average percentage is 72.1%.

A close analysis of the data reveals that sodium chloride is the most easily recognized compound, even at the lowest concentration; the system gives good results with citric acid, especially at the highest concentrations, while the performance with glutamic acid improves with the increase of concentration, though extremely high values of recognition are never reached. Very poor performance is obtained with sodium dehydrocholate and glucose, even in the case of the highest concentrations. Furthermore, it can be seen that often the wrong predictions assign the sample to totally different compounds.

If the system had to be used for a real application, a more realistic assessment of the performances would be obtained by the leave-1-day-out approach, which does not compensate for the time drift. Results obtained with this approach are reported in Table 5.

The average percentage of correct predictions is 53.4%, as expected lower than the value obtained with the Venetian blind approach. These results confirm the previous findings, with very good predictions for sodium chloride (70.0% of correct predictions even at low concentration) and citric acid (98.5% of correct predictions at the highest concentration), acceptable results for sodium glutamate (58.0% at the highest concentration) and poor results for bitter and glucose. It is also interesting to study the performance of the single sensors and variables, in order to try to obtain the same (or better) predictive ability with a simpler instrument. Variables 2, 4, 6 and 7 (1M, 3M, 1F and 2F) produce 66.7% correct predictions in cross-validation with the Venetian blind.

Table 5
Diagonal of the confusion matrix after the leave-1-day-out validation

	Correct predictions (%)
CAL	26.7
CAM	90.4
CAH	98.5
SDL	45.1
SDM	20.6
SDH	42.8
GL	51.7
GM	0.8
GH	25
SCL	70
SCM	88.9
SCH	95.3
GAL	37.5
GAM	49.5
GAH	58
Mean	53.4

While there are good reasons to criticize the concept of the five basic tastes (Delviche, 1996), and we are still far from being able to instrumentally reproduce human perception, the results demonstrate the feasibility of using a composite array along with a rigorous experimental testing scheme to discriminate simple soluble compounds.

4. Conclusion

In this work an impedentiometric electronic tongue based on a composite array has been used to classify different solutions eliciting five basic tastes (sodium chloride, citric acid, glucose, glutamic acid and sodium dehydrocholate for salty, sour, sweet, umami and bitter, respectively) at three concentration levels comprising the human perceptive range. Novel aspects of this work are the use of a composite sensor array, i.e. an array consisting different materials characterized by different response mechanisms, and the acquisition of the electrical impedances at a frequency of 150 Hz as transduction signals. To eliminate artifacts due to over-fitting of data, an intensive number of test over a long time period were performed, using random measurement campaigns. Solution identification was performed using PCA for detecting and removing outliers and LDA for pattern classification. An average recognition percentage of 72.1% was obtained. The compounds best identified were salty, acid and umami, demonstrating the feasibility of using electrical impedance to monitor the response of a sensor array in liquids, whereas bitter and sweet gave rather poor results. The authors are confident that solution discrimination can be improved through the use of a larger and more varied array and more sophisticated data processing methods.

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