CHAPTER 2

Chemometric Brains for Artificial Tongues

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Contents

Abstract

The last years showed a significant trend toward the exploitation of rapid and economic analytical devices able to provide multiple information about samples. Among these, the so-called artificial tongues represent effective tools which allow a global sample

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characterization comparable to a fingerprint. Born as taste sensors for food evaluation, such devices proved to be useful for a wider number of purposes.

In this review, a critical overview of artificial tongue applications over the last decade is outlined. In particular, the focus is centered on the chemometric techniques, which allow the extraction of valuable information from nonspecific data. The basic steps of signal processing and pattern recognition are discussed and the principal chemometric techniques are described in detail, highlighting benefits and drawbacks of each one. Furthermore, some novel methods recently introduced and particularly suitable for artificial tongue data are presented.

I. INTRODUCTION

The evolution of food processing from small-scale craft production to an industrial scale of production of food created several new needs. Among these, the possibility of performing an analytical supervision, monitoring every production stage, is one of the most important. Such control may address two main issues. The first one is a research-and-development aim: to study all the factors involved in the process, in order to ascertain the optimal production conditions, which are able to provide a product with the highest consumer-satisfaction rate while keeping costs and time as low as possible. The second purpose concerns the normal production phase and involves continuous monitoring of the manufacturing processes during regular daily manufacturing for the ability to determine, in real time, any eventual faults. Also in this case, the final aim is to achieve significant cost and time savings.

Another important analytical requirement concerns the quality control of the incoming ingredients and of the outgoing finished product. Quality, according to Taguchi (1986), is intended as the minimization of the variability about a target value, as indicated in Fig. 2.1.

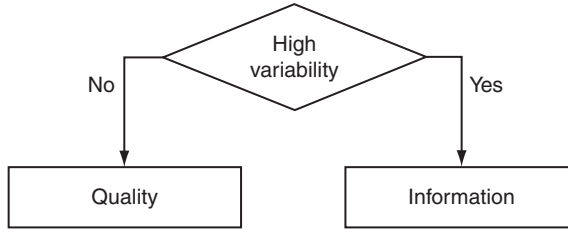

FIGURE 2.1 Implications of variability amount.

All these issues require the execution of analyses with a very high frequency. As a result, the demand for instrumentation able to provide chemical and physical information rapidly and cheaply is constantly increasing. Furthermore, analytical techniques should be performed without destruction of samples or, at least, with a minimal sample consumption, thus facilitating at-, on-, and in-line implementation. They should be easy to perform, automatable, and robust.

From the consumer's point of view as well, controls on food products are needed, from a food safety and traceability perspective. The problem of adulteration and falsification of food has ancient roots which date back to the Roman Age and continue, through the Middle and Modern Ages, till nowadays: the deployment of new analytical tools for fraud detection has caused a parallel progress in the adulteration procedures, which has gradually evolved from coarse and rudimentary systems to highly sophisticated and scarcely detectable strategies.

For these reasons, in the last decades, there has been an increasing attention, among national and international authorities, toward food safety and authenticity problems: the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) played a chief role in regulation and control.

During the years 1961–1963, the Codex Alimentarius Commission (CODEX) has been instituted as an intergovernmental organization devoted to establish international food standards, such as hygienic practice policies, food labeling codes, limits for additives, contaminants, pesticides, veterinary drug residues, and so on (Lupien, 2002). The CODEX efforts over the past fifty years have led to far more than 200 standards for different food products and guidelines for labeling, sampling, and analysis (http://www.codexalimentarius.net/).

Also the consumer's needs—exactly like the manufacturer's ones—require rapid, effective, low-cost, and nondestructive analytical techniques, in order to accomplish a widespread control and achieve broader quality assurances saving the costs.

Instrumental devices able to address all these requirements have been defined by Valcárcel and Cárdenas (2005) as ''vanguard analytical strategies''. Frequently, such methods provide nonspecific information, which provides a global sample characterization analogous to a fingerprint and sometimes can also be used to identify or quantify specific analytes in a sample. Such nonspecific techniques, in comparison with the classical specific analytical approaches, usually offer several advantages, the most significant of which are high speed, low costs, no or minimal sample pretreatments, no destruction of the sample, no requirement for highly skilled personnel, the possibility of at-, on-, and in-line deployments, automation, and transportability. Typical examples are constituted by spectrophotometric techniques and by the so-called artificial noses and

artificial tongues. These latter devices have been developed with the initial aim of reproducing the mammalian sensorial evaluation of samples in many potential application fields: analysis of bioactive and/or toxic substances, like pharmaceutical formulations and environmental samples; replacement of human sensory panels, overcoming some related hurdles like limitations in the number of samples assessable in each test session, high costs, and low repeatability and reproducibility of the responses.

The sensations in mammals result from neural processing of thousands of stimuli coming from many different peripheral sensors. These two main elements—inputs from terminal sensors and central processing—are exactly what the sensory-like analytical techniques aim at reproducing: the sensorial stimulus is replaced by the response of an instrumental sensor or a sensor array suitably arranged, while the elaboration stage consists of proper processing of instrumental data (see Fig. 2.2). Since both sensorial stimuli and brain elaboration in nature are multiple and simultaneous, in order to reproduce such mechanisms also the instrumental data processing should be conducted in a multivariate way.

In parallel, during recent years, several applications of artificial noses and tongues were demonstrated to be suitable not only for a sensory-like evaluation but for a wider-ranging characterization of the samples. Non-specific analytical responses, in fact, may provide information about the

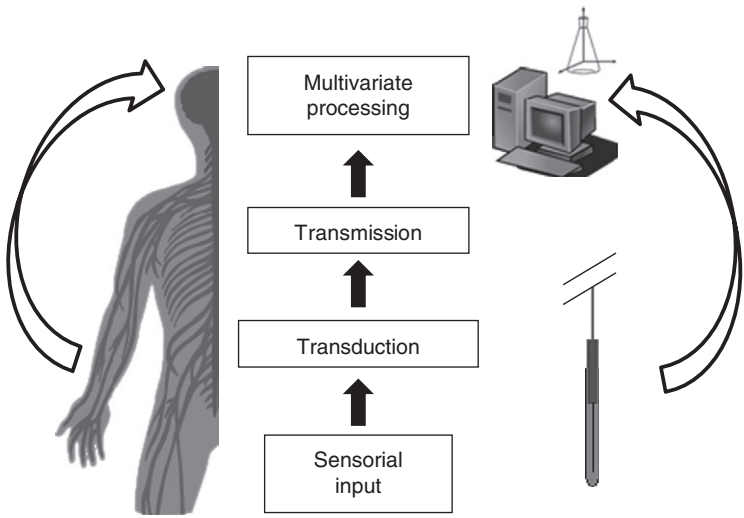

FIGURE 2.2 Parallelism between sensorial mechanisms in mammals and sensory-like analytical techniques.

geographical origin of a product, the quality of the ingredients employed, the type of manufacturing process, and so on. As a consequence of this big potential, the application fields of artificial noses and tongues have been considerably extended, embracing a number of sectors other than food science.

Anyway, whatever the instrument and the application, an aspect is unvarying: the only way to obtain valuable information from these analytical systems is to process data by means of multivariate tools, that is to say, to make use of chemometrics.

Obviously, the quality of the final information depends both on the quality of the sensors and on that of the brain.

In this review, an in-depth overview is presented, tracing an outline of the chemometric techniques most widely applied in the relatively brief history of artificial tongues, highlighting benefits and drawbacks of each one. Furthermore, some chemometric methods recently introduced and particularly suitable for artificial tongue data processing are discussed.

II. TERMINOLOGY

The term artificial tongue is used in two main branches of science. The first one concerns the neurophysiological studies aimed at developing perceptual supplementation devices, with biomedical engineering applications to human disabilities. The second utilization of the term artificial tongue concerns, instead, the laboratory analytical instruments used in combination with chemometric techniques to obtain complex information (often sensory-like, but not only) on samples. As for this latter meaning, also the synonymous electronic tongue is frequently used, particularly for electroanalytical devices.

In order to better describe the utilization purposes, some authors proposed a distinction between electronic tongues and taste sensors: the former term should have a wider meaning, embracing all the possible applications, while the latter should exclusively refer to sensory-like evaluations.

A sensor is a device able to respond to the presence of one or many given substances in a more-or-less selective way, by means of a reversible chemical interaction: it may be employed for qualitative or quantitative determinations (Cattrall, 1997). All sensors are composed of two parts: the responsive region and the transducer. The responsive region is responsible for sensitivity and selectivity of the sensor, while the transducer converts energy from one to another form, providing a signal which is informative about the system analyzed. Usually, basic signal-processing electronics, and control and display units complete the device.

Nowadays, the signals are almost always digitally recorded and stored for subsequent processing, which involves the application of chemometric pretreatment and pattern recognition tools.

A wide number of sensor types have been described in the literature, from optical to mass spectrometry-based devices, but the sensors most commonly used in artificial tongues are electrochemical.

III. HISTORY

The history of electrochemical sensors began in the thirties of the twentieth century, when the pH-sensitive glass electrode was deployed, but no noteworthy development was carried out till the middle of that century. In 1956, Clark invented his oxygen-sensor based on a Pt electrode; in 1959, the first piezoelectric mass-deposition sensor (a quartz crystal microbalance) was produced. In the sixties, the first biosensors (Clark and Lyons, 1962) and the first metal oxide semiconductor-based gas sensors (Taguchi, 1962) started to appear.

Nevertheless, it was only starting from the seventies and the eighties that the technology began to deploy highly sophisticated devices, which are continuously evolving toward an increasing miniaturization and the development of portable instrumentation.

The first utilization of the term artificial tongue dates to 1978, when H.W. Harper and M. Rossetto presented an apparatus based on conductance measurements able to mimic the taste stimulus delivery systems (Harper and Rossetto, 1978). This pioneering work has represented an isolated study for several years. The first example of a factual taste sensor was developed, in fact, by Toko and coworkers in 1990 (Hayashi *et al.*, 1990; Toko *et al.*, 1990). It was based on ion-sensitive lipid membranes and it was claimed to be able to respond to the basic tastes of the human tongue: sour, sweet, bitter, salt, and *umami*.

The term electronic tongue was created in 1996, at the Eurosensors X Conference by A. Legin, C. Di Natale and coworkers (Di Natale *et al.*, 1996; Legin *et al.*, 1996).

Starting from the nineties, the number of original research papers on ISI journals has been gradually increasing for almost one decade, showing the maximum expansion between 2003 and 2008, as it is noticeable in the bar-plot of Fig. 2.3.

A few commercial instruments have become available, for instance, from Insent Inc. (Atsugi-chi, Japan) and Alpha MOS (Toulouse, France). They are potentiometric devices for taste sensing, mainly used in the pharmaceutical sector.

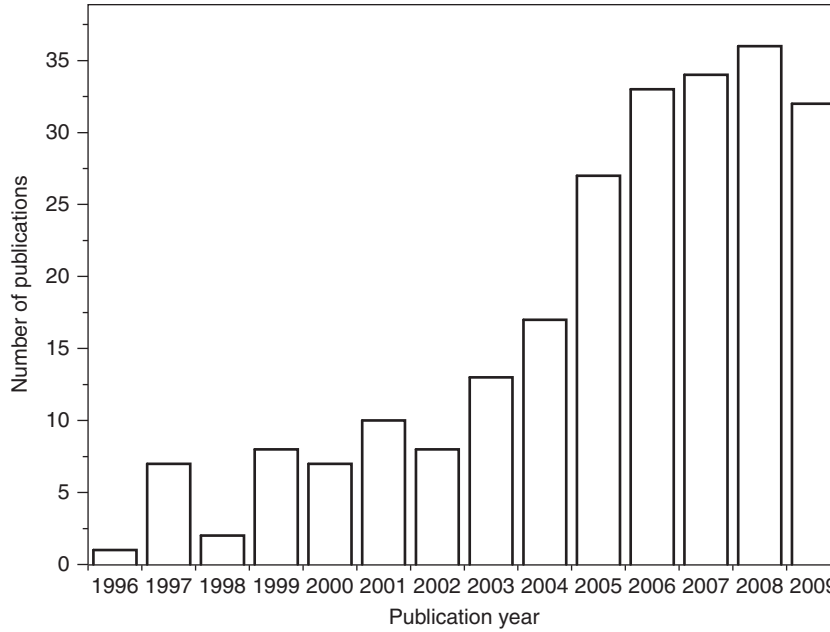

FIGURE 2.3 Trend of electronic tongue original research papers over the period 1996–2009. Data obtained from a literature search using SciFinder Scholar.

IV. MAIN APPLICATION SECTORS

Taste sensors, and, more generally, artificial tongues may work in two main modalities: qualitative and quantitative. The qualitative approach usually consists in the identification and classification of samples on the basis of some particular property, like, for instance, the taste or the geographical origin. Quantitative applications, instead, may be either the simultaneous calibration of multiple analytes or the prediction of sensorial attributes and/or chemical-physical parameters. Not rarely, in the same study, both of the approaches are followed: data collected with the same instrument from the same samples are treated by different chemometric strategies to obtain qualitative and quantitative information, respectively. For example, the nonspecific voltammetric profiles recorded in wine samples may be used both for characterizing wines on the basis of their provenance and for quantifying parameters of oenological interest.

From examination of almost 230 papers published in ISI journals over the period 1996–2009, it emerges that the many sectors in which artificial tongues have found applications go from the industrial plant process-monitoring to biomedical and clinical studies (see Fig. 2.4). As for this latter field, a number of sensors for the determination of several clinical

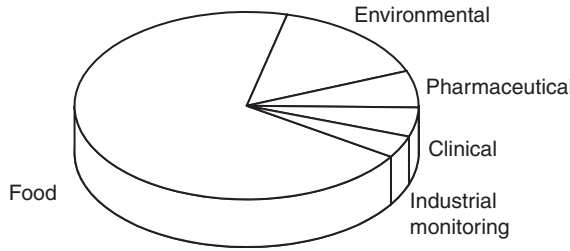

FIGURE 2.4 Main application sectors of electronic tongues. Data obtained from a literature search over the period 1996–2009, using SciFinder Scholar.

parameters such as urea, glucose, and triglycerides and for the diagnosis of several diseases by nonspecific analysis of biological liquids have been deployed (Ciosek *et al.*, 2008; Mottram *et al.*, 2007; Sangodkar *et al.*, 1996; Sohn *et al.*, 2005; Sukeerthi and Contractor, 1999; Wang *et al.*, 2007).

Another considerable employment sector is that of environmental analyses, particularly in relation to the detection of heavy metal traces and various organic contaminants in water, such as pesticides and residuals from industrial plants (Aoki *et al.*, 2009; Calvo *et al.*, 2008; Carvalho *et al.*, 2007; Constantino *et al.*, 2004; Cortina *et al.*, 2006; Di Natale *et al.*, 1997; Gutes *et al.*, 2005; Gutierrez *et al.*, 2008; Hu *et al.*, 2008; Ipatov *et al.*, 2008; Kulapina and Mikhaleva, 2005; Makarova and Kulapina, 2009; Martinez-Manez *et al.*, 2005; Men *et al.*, 2004, 2005; Mikhaleva and Kulapina, 2006; Mourzina *et al.*, 2001; Olsson *et al.*, 2008; Turek *et al.*, 2009; Valdes-Ramirez *et al.*, 2009).

A further widespread area of utilization, particularly of the taste sensors, is represented by pharmaceutical technology studies: in fact, in order to enhance patient compliance to oral drugs, there is an incessant search for pharmaceutical formulations able to mask the bitter and/or disagreeable taste which characterizes many active principles; however, since such formulations are biologically active, traditional taste assessments by means of panel tests are a problematical task. For this reason, instrumental devices, such as artificial tongues, able to provide a response correlated with human taste perception are enormously advantageous (Agresti *et al.*, 2008; Kayumba *et al.*, 2007; Krishna Kumar, 2006; Legin *et al.*, 2004; Li *et al.*, 2007a; Lorenz *et al.*, 2009; Sadrieh *et al.*, 2005; Takagi *et al.*, 2001; Tokuyama *et al.*, 2009; Zheng and Keeney, 2006).

Anyway, the principal use of artificial tongues is within the food sciences. The applications concern almost exclusively liquid food: mainly wine (about 18% of the studies examined), fruit juices (almost 15%), mineral water (about 13%), followed by infusions like tea and coffee, soft drinks, milk, beer, and other alcoholic beverages. All these liquid foods are characterized by both low-viscosity and high-polarity values,

which provide them a suitable electrical conductivity. Instead, low-polarity and high-viscosity liquids, such as vegetable oils and honey, are rather less studied, due to the intrinsic difficulties in performing electroanalytical measurements in such media. Only a few examples of application to solid foods, like vegetables and fish, can be found in the literature: in all these cases, the analytical protocol includes more-or-less complex sample pretreatment stages (Bengtsson *et al.*, 2007; Beullens *et al.*, 2006; Ciosek *et al.*, 2006c; Rudnitskaya *et al.*, 2006a,b). Some, especially early, studies based on electronic tongues explored the possibility of differentiating liquid foods with very different natures-like, for example, mineral water, wine, milk, coffee, and so on, as in the example of Fig. 2.5 (Ciosek and Wroblewski, 2007; Gallardo *et al.*, 2005; Vlasov *et al.*, 2000).

Such studies are of limited practical interest since, in everyday life, it is possible—for example—to easily distinguish coffee from fruit juice without making use of electroanalysis and chemometrics. Nevertheless, in many cases, these studies aimed at assessing the potential of novel sensors and served as the bases for subsequent research work and technical improvements.

Examples of more advanced applications are, for instance, the discrimination among different brands of the same food (Ciosek and Wroblewski, 2006; Ciosek *et al.*, 2004a,b, 2005, 2006a–d, 2007, 2009; Legin *et al.*, 1999; Martina *et al.*, 2007; Riul *et al.*, 2003a,b; Winquist *et al.*, 2000).

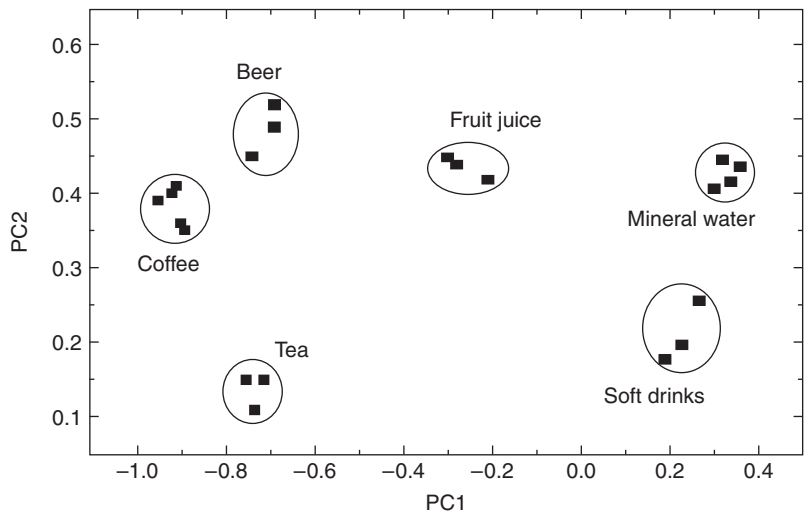

FIGURE 2.5 Differentiation of beverages of very different nature by means of an electronic tongue based on potentiometric sensor arrays (reproduced from Vlasov *et al.*, 2000, with permission).

Other interesting applications arise from studies concerning vegetable-derived foods, where the differentiation is carried out according to the botanical variety or cultivar of the plant involved (Beullens *et al.*, 2008; Dias *et al.*, 2008; Kantor *et al.*, 2008; Rudnitskaya *et al.*, 2006b). In other cases, the attention is focused onto the geographical origin (Buratti *et al.*, 2004; Cosio *et al.*, 2006; Oliveri *et al.*, 2009).

A considerable part of the studies based on artificial tongues within food sciences aims at evaluating food taste properties, like bitterness and astringency and/or typical descriptors of sensorial analysis (Buratti *et al.*, 2006; Buratti *et al.*, 2007; Fung *et al.*, 2004; Legin *et al.*, 2003; Li *et al.*, 2007b; Puech *et al.*, 2007; Rudnitskaya *et al.*, 2009a; Scampicchio *et al.*, 2006). Such applications usually present worse results than those obtained for the prediction of chemical quantities and objective chemical–physical descriptors. The reasons for this inferiority do not come—or, at least, not only—from instrumental inadequacy, but from the poor repeatability and reproducibility which inexorably characterize sensorial analyses, in spite of the accurate training of the panels and their considerable remuneration.

In some interesting studies, artificial tongues are employed to evaluate the effect of a number of process factors on the quality of the finished products (Esbensen *et al.*, 2004; Rollm de Moura *et al.*, 2007; Rudnitskaya *et al.*, 2009b) or the effects of storage time and conditions (Apetrei *et al.*, 2007; Cosio *et al.*, 2007; Kantor *et al.*, 2008; Parra *et al.*, 2006a; Rodriguez-Mendez *et al.*, 2007).

Other important research activities aim at carrying out the setting up of analytical methods specifically committed to fraud detection (Chen *et al.*, 2008; Dias *et al.*, 2009; Legin *et al.*, 2005; Parra *et al.*, 2006b).

V. ANALYTICAL TECHNIQUES

Several analytical devices have found application as test sensors or, more generally, as electronic tongues for characterizing foods or food ingredients, being able to provide information related to the human sensorial perception or to other important features. There are some examples of electronic tongues based on optical techniques as well as on mass measurements, but the analytical methods that have been most widely exploited in this field are, without any doubt, the electrochemical ones, as shown in Fig. 2.6.

Within the electroanalytical sector, potentiometry and voltammetry are the principal methods applied in electronic tongue studies, followed by impedance spectroscopy.

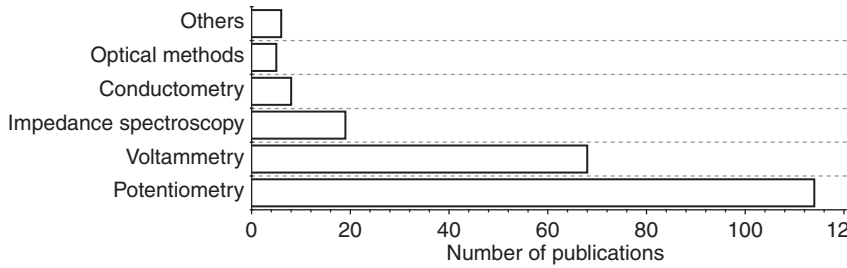

FIGURE 2.6 Main analytical techniques applied as electronic tongues. Data obtained from a literature search over the period 1996–2009, using SciFinder Scholar.

A. Potentiometry

Figure 2.6 shows that almost one-half of the artificial tongues described in the scientific literature of the last decade are based on potentiometric devices.

Potentiometry is a technique traditionally employed for the quantification of ions in a liquid solution. It is a static electroanalytical method, that is, there is no current flow inside the measurement cell ($i = 0$). The measurement cell is constituted by two electrodes which are immersed in the solution containing the analytes. A voltmeter measures the potential difference between the two electrodes, which is a function of the concentration (actually, the activity) of the analytes, as described by the well-known Nerst's equation (Kissinger and Heineman, 1996).

The most common analytical applications require one of the two electrodes to be characterized by an unchanging potential, known and independent of the characteristics of the solution being analyzed. Such a device is called the reference electrode. One of the most commonly used is the Ag/AgCl electrode, which consists of a silver wire coated with silver chloride and immersed into a solution saturated by chloride ions; a porous plug serves as a connection bridge with the outer solution.

The other electrode, called the indicator electrode, is directly involved in the interaction with the analyte and it is usually selective toward particular species. A huge number of different devices have been deployed with various characteristics, for a lot of applications. Typically, in the potentiometric electronic tongues, several selective electrodes are employed together, constituting a sensor array, with the aim of providing a global characterization of the sample, similar to the evaluation made by human senses. This concept has been synthesized by Toko with the expression global selectivity (Toko, 1998): in the electronic tongue philosophy, the explicit quantification of single analytes is not the important aspect. On the contrary, it is often an implicit step in determining sensorial and other particular properties, which may be a function of the proportions between hundreds or even thousands of species in a sample.

B. Voltammetry

Voltammetry is the second most utilized technique for electronic tongue devices (see Fig. 2.6). It is a dynamic electroanalytical method, that is, a current flow passes through the measurement cell ($i \neq 0$). Voltammetry consists of the measurement of current at a controlled potential: constant or, more frequently, varying. In the classic three-electrode cell configuration, the current flows between two electrodes, called working and counter (or auxiliary) respectively, while the potential is controlled between the working and a third electrode, the reference (Kissinger and Heineman, 1996).

The signal recorded is generally the current versus potential profile, which is called voltammogram.

In voltammetry, different measurement modalities can be deployed on the basis of the nature of the potential versus time variation.

In chronoamperometry, which is employed in a few electronic tongue systems (Cortina *et al.*, 2008; Han *et al.*, 2004), the potential is kept constant, while the current variations, resulting from faradic processes occurring at the electrode, are monitored as a function of time.

In linear sweep voltammetry, the potential is varied linearly versus time, and current peaks are registered in correspondence to oxidation or reduction (depending on the potential variation verse) of the analytes. The potential value associated to a peak is characteristic of the specie being oxidizing or reducing, while the peak height can be employed for quantitative purposes.

Two closely related techniques are staircase and square-wave voltammetry: in the first modality, the potential is varied stepwise versus time; in the second modality, a pulse square-wave is superimposed onto the staircase potential variation. In the differential acquisition mode, the current is measured immediately before each potential change, and the current difference is plotted as a function of potential. This way, the effect of the charging current can be decreased.

Another evolution from the linear sweep mode is cyclic voltammetry, namely, a sequential combination of two (or more) linear sweep potential scans in the opposite direction: for this reason, the current versus potential response supplies information about the reversibility of redox systems.

C. Impedance spectroscopy

Impedance spectroscopy is a versatile electrochemical tool, helpful to characterize the intrinsic dielectric properties of various materials. The basis of this technique is the measurement of the impedance (opposition to alternating current) of a system, in response to an exciting signal over a range of frequencies (Bard and Faulkner, 2001).

Impedance spectroscopy may provide quantitative information about the conductance, the dielectric coefficient, the static properties of a system at the interfaces, and its dynamic changes due to adsorption or charge-transfer phenomena. Since in this technique an alternating current with low amplitude is employed, a noninvasive observation of samples with no or low influence on the electrochemical state is possible.

VI. CHEMOMETRICS

Chemometrics is a chemical discipline born for interpreting and solving multivariate problems in the field of analytical chemistry. Svante Wold used for the first time, in 1972, the name chemometrics for identifying the discipline that performs the extraction of useful chemical information from complex experimental systems (Wold, 1972).

In 1997, D.L. Massart suggested the following definition: ''Chemometrics is a chemical discipline that uses mathematics, statistics and formal logic (a) to design or select optimal experimental procedures; (b) to provide maximum relevant chemical information by analyzing chemical data; and (c) to obtain knowledge about chemical systems'' (Massart *et al.*, 1997).

The first of these aims is addressed by multivariate design of experiments (MDOE), while pattern recognition techniques deal with the remaining objectives. Since the quality of a result depends on the distribution of the experiments in the experimental domain, the main purpose of MDOE is to select the set of experiments resulting in the highest possible information amount, given the experimental limitations and the budget available (i.e., the maximum number of performable experiments). With the tools of MDOE, it is possible to find optimal conditions for multiple factors, considering their interactions, within almost every type of multivariate procedure, from analytical methods to organic synthesis and industrial processes. Despite its considerable effectiveness and the easy-to-understand concepts on which it is based, MDOE is applied in a minimal number of scientific studies. MDOE techniques are employed in just 5 over the nearly 230 original research papers on electronic tongues published during the last decade and examined for this review. (Holmin *et al.*, 2004; Labrador *et al.*, 2009; Li *et al.*, 2007a; Rollm de Moura *et al.*, 2007; Rudnitskaya *et al.*, 2009b).

On the other hand, pattern recognition tools are widely employed for processing data in the field of electronic tongues and, more generally, of artificial senses. Nowadays, a large number of chemometric techniques, which are schematized in Fig. 2.7, are available, giving the

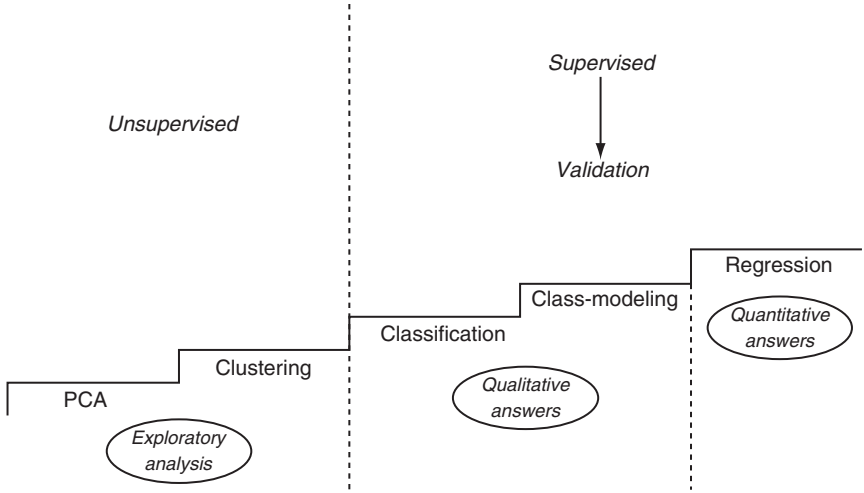

FIGURE 2.7 Main steps of pattern recognition.

possibility of achieving several different results from the analysis of a data set, namely:

- recognition of the presence of structures (clusters, correlation) among the objects and/or the variables studied (exploratory analysis, unsupervised)
- deployment of mathematical models for prediction of qualitative responses (classification and class-modeling analysis, supervised)
- deployment of mathematical models for prediction of quantitative responses (regression analysis, supervised).

Several very important accessory tools, for example for data preprocessing and variable selection, complete the chemometric pattern recognition arsenal.

As the definition says, a model is a description of a real phenomenon performed by means of mathematical relationships (Box and Draper, 1987). It follows that a model is not the reality itself: it is just a simplified representation of reality. Chemometric models, different from the models developed within other chemical disciplines (such as theoretical chemistry and, more generally, physical chemistry), are characterized by an elevated simplicity grade and, for this reason, their validity is often limited to restricted ranges of the whole experimental domain.

Nevertheless, chemometric models are not developed with the aim of supporting a theory or describing a phenomenon from a general point of view, but with the aim of obtaining answers for particular real problems. Therefore, if the validity range of a model corresponds to the region of

practical utility and interest, it means that such a model is functional to the aim for which it has been developed.

George E.P. Box synthesized all these concepts in a sentence: ''all models are wrong, but some are useful'' (Box and Draper, 1987).

From this perspective, the ultimate aim of chemometrics should be the development of useful models. Such a target can be accomplished only by means of a deep chemical knowledge of the problem to be solved, of the type of data to be handled, and finally, of the tools for multivariate analysis.

The development of models always requires these models to be carefully validated (see Fig. 2.7), by means of a proper validation strategy, in order to provide information about the actual validity and usefulness in relation to the problem studied. If not validated, a model is not exploitable in the practice, since the reliability of its outcomes is completely unknown.

A. Multivariate design of experiments

MDOE embraces a number of tools which permit experiments to be conducted in the most efficient possible way, achieving several interesting results such as screening of the important factors, optimization of manufacturing and analytical procedures, minimization of costs and pollution, and robustness testing of products and processes.

When a new analytical method is being developed, MDOE is extremely useful, since it is able to identify the key experimental factors and the optimal experimental conditions, by performing a minimal number of experiments, that is, with the lowest possible effort and costs.

The first stage of any experimental design is the problem formulation, a basic step in which the objectives and thus the response variable to be optimized should be defined. After that, it is essential to identify all the factors that might have an influence on the selected responses, and for each factor, variability levels that take into account eventual constraints.

Then, a screening method may identify which factors have a significant effect on the response.

At this point, a model has to be postulated based on the significant factors: the main types are linear, linear with interactions, and quadratic polynomial models. The next step is the definition of the experimental plan, which is closely dependent on the model chosen and on the number of factors.

When few factors (f from two to four) are studied, the full factorial design is the most common approach. The full factorial scheme is the basis for all classical experimental designs, which may be used in more complex situations. For a general two-level full factorial design, each factor has to be considered at a low level (coded as -1) and a high level

(coded as +1), whose values are defined by the operator on practical bases. The experimental plan derives from all the possible level combinations (2^f) for all the factors studied: in the case of two factors, the number of experimental conditions is 2^2 and the possible combinations are low–low, low–high, high–low, and high–high; they are located at the vertices of a square and, consequently, they explore the whole experimental domain. In the case of three factors, the number of experimental conditions is 2^3, located at the vertices of a cube, as shown in Fig. 2.8. From the experiments performed under each condition tested, the response variable is measured and the values obtained are used to build the model, which usually also estimates the interactions between variables (i.e., the effect that the value of one factor has on changing other factors). The complete mathematical model is therefore the following:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (1)$$

where Y is the response variable and X_1, X_2, and X_3 are the three factors. With just eight experiments, it is possible to estimate a constant term (b_0), the three linear terms (b_1, b_2, and b_3), the three two-term interactions (b_{12}, b_{13}, and b_{23}), and the three-term interaction (b_{123}). Usually, the model is validated by predicting the response value in the point corresponding to the center of the experimental domain (the center point in Fig. 2.8).

When the number of factors is higher and/or when the model postulated is more complex, more advanced strategies can be employed, such

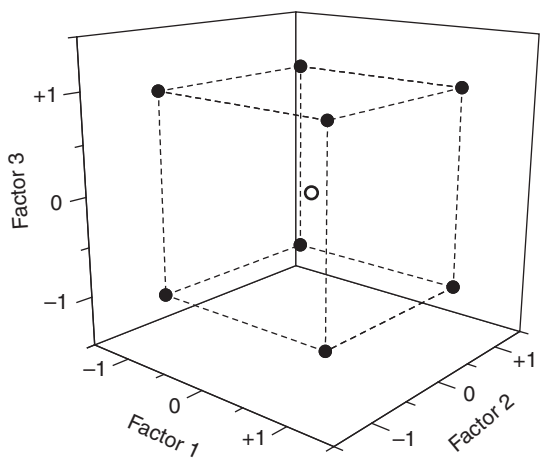

FIGURE 2.8 Coded levels for the experiments required by a two-level full factorial design with three factors.

as the fractional factorial design, central composite design, and D-optimal design, which allows the choice of a set of informative experiments by way of optimization criteria (Box *et al.*, 1978; Leardi, 2009).

B. Preprocessing

In the case of the complex analytical signals arising from artificial tongue instruments, a number of preprocessing tools may be employed for three main purposes, namely:

- elimination or reduction of random noise
- elimination or reduction of unwanted systematic variations
- data reduction or compression.

Unwanted systematic variations may be due to instrumental hurdles, to the experimental conditions and/or to physical characteristics of the samples. In general, it might be advantageous to avoid unwanted signal variations by improving the experimental settings, but this approach is not always feasible. Furthermore, in many cases, the effort that is needed (complex sample pretreatments, accurate temperature control, and so on) may be so high that it becomes incompatible with the ideal characteristics of a vanguard analytical system, which has to be cost and time saving and as simple as possible (Valcárcel and Cárdenas, 2005; Vlasov *et al.*, 2005). For these reasons, when it is possible, it may be preferred to remove the signal variations afterward, by data preprocessing, following the saying ''math is cheaper than physics'' (Kohler *et al.*, 2009).

1. Row preprocessing

Row preprocessing acts on each single signal, independent of the other ones, and the result is the correction of systematic differences among signals.

Figure 2.9 and Table 2.1 summarize the effects of a number of common row pretreatments, discussed below, in correcting for unwanted signal variations such as addictive (baseline shifts), multiplicative (baseline drifts), and global intensity variation effects.

Each signal (y_i), defined by V variables (e.g., the current values in a voltammogram), is corrected individually by subtracting its mean value ($\bar{y}_i$) from each single value ($y_{i,v}$). The values of the row-centered signal ($y_{i,v}^*$) are obtained by

$$y_{i,v}^* = y_{i,v} - \bar{y}_i \tag{2}$$

After transformation, the mean value of each signal is equal to 0: for this reason, row centering removes systematic location differences, namely baseline shifts, from a set of signals, as it is shown in Fig. 2.9A.

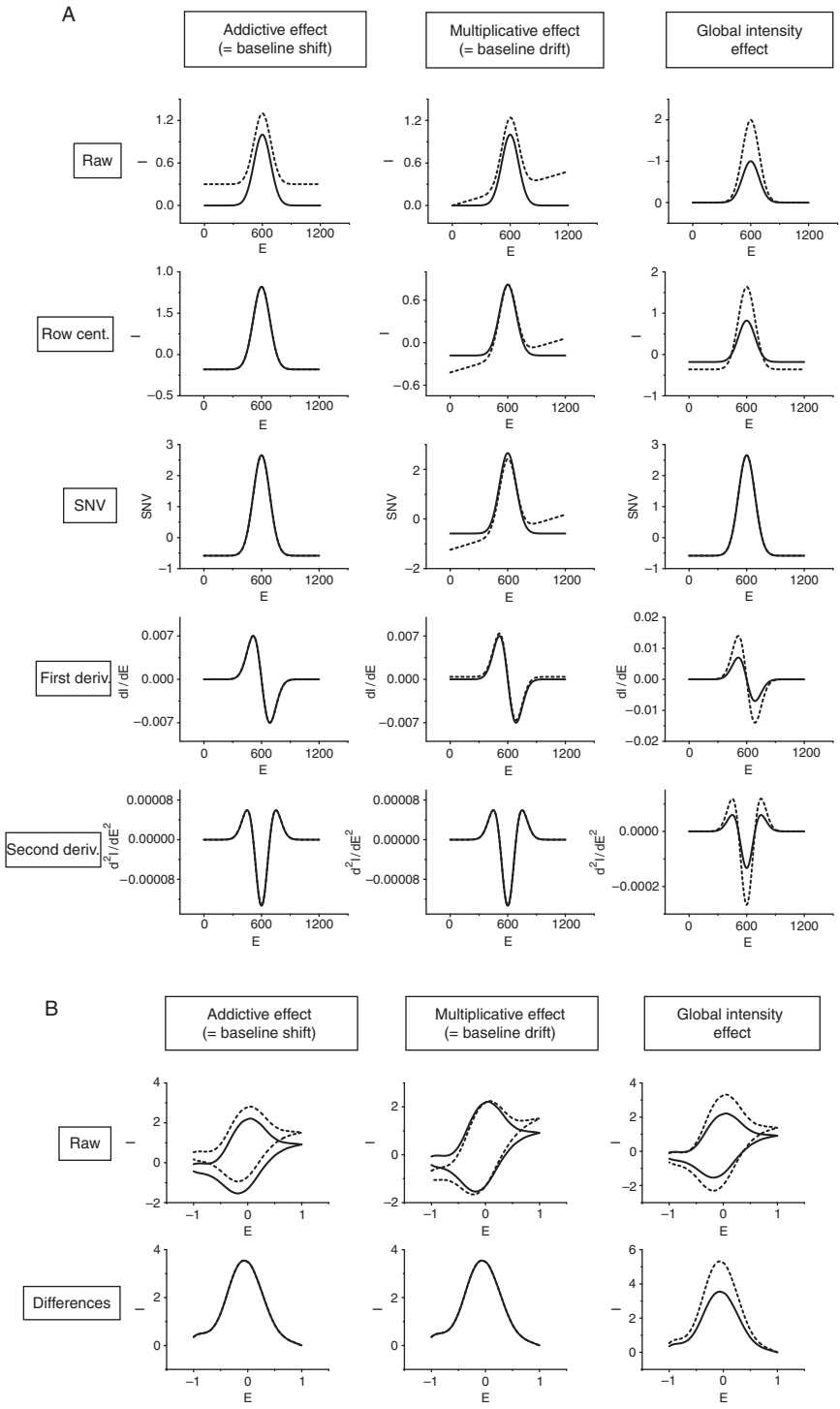

FIGURE 2.9 Effectiveness of a number of row pretreatments in eliminating addictive, multiplicative, and global intensity effects.

TABLE 2.1 Summary of the corrections for unwanted signal variations, practicable by application of appropriate row pretreatments

	Addictive	Multiplicative	Intensity
Row centering	✓		
SNV	✓		✓
First derivative	✓		
Second derivative	✓	✓	
Differences	✓	✓	

a. Standard normal variate transform Standard normal variate transform (SNV), or row autoscaling, is a mathematical method for signal transformation, particularly applied in spectroscopy, which is useful to remove slope variations and to correct for both baseline shifts and global intensity variations (Barnes *et al.*, 1989) (see Fig. 2.9A). Each signal (y_i) is row-centered, as described above, and then scaled by dividing the single values by the signal standard deviation (s_i). The values of the SNV transformed signal are obtained by

$$y^*_{i,v} = \frac{y_{i,v} - \bar{y}_i}{s_i} \tag{3}$$

After transformation, each signal presents a mean value equal to 0 and a standard deviation equal to 1: both location and dispersion systematic differences are corrected.

SNV has the peculiarity of potentially shifting informative regions all along the signal range, so that the interpretation of the results referring to the original signals may be deceiving (Fearn, 2009).

b. First and second order derivation after smoothing The numerical differentiation of digitized signals has many uses in analytical signal processing. It allows a signal resolution enhancement, increasing the apparent resolution of overlapping peaks, accentuates small structural differences between nearly identical signals, and corrects for baseline shifts and drifts, depending on the derivation order, as shown in Fig. 2.9A (Taavitsainen, 2009).

In particular, the first derivative of a signal $y = f(x)$ is the rate of change of y with x (i.e., $y' = dy/dx$), which can be interpreted as the slope of the tangent to the signal at each point. It returns null segments in correspondence to constant bands of the original signal and provides a correction for addictive effects.

The second derivative can be considered as a further derivation of the first derivative ($y'' = d^2y/dx^2$); it represents a measure of the curvature of the original signal, that is, the rate of change of its slope. The second derivative returns null segments in correspondence to bands characterized by a constant slope in the original signal and provides a correction for both addictive and multiplicative effects.

A disadvantageous consequence of derivation may be an enhancement of the noise, which is usually characterized by high-frequency slope variations. To overcome this hurdle, signals are firstly smoothed, often by using the Savitzky–Golay algorithm, which is a moving window averaging method (Savitzky and Golay, 1964). A third-degree smoothing polynomial and a window size of 5–15 datapoints are suggested.

c. Difference between forward and backward currents Computing the difference, point by point, between the forward and the backward current values evaluated at the same potential, is a peculiar pretreatment for cyclic voltammograms (Oliveri *et al.*, 2009), that is able to eliminate both baseline shifts and drifts (see Fig. 2.9B), and therefore improve the availability of useful information in data analysis. The number of variables in the resulting data matrix is half of the original one.

2. Column preprocessing

Column preprocessing corrects each variable individually and the result is the correction of systematic differences among variables.

a. Column centering Each variable defining a set of signals (e.g., the current values at different potentials in a set of voltammograms) is corrected individually by subtracting its mean value ($\bar{y}_v$) from each single value ($y_{i,v}$). The column-centered values ($y^*_{i,v}$) are obtained by

$$y^*_{i,v} = y_{i,v} - \bar{y}_v \tag{4}$$

After transformation, the mean value of each column is equal to 0, so that systematic location differences among variables are eliminated.

b. Column autoscaling Column autoscaling is often a default data pretreatment in chemometrics, particularly in the case of variables of different nature. Each variable is corrected individually by subtracting its mean ($\bar{y}_v$) from each of its values and then dividing by its standard deviation (s_v). The autoscaled values are dimensionless and are computed by

$$y^*_{i,v} = \frac{y_{i,v} - \bar{y}_v}{s_v} \tag{5}$$

This column preprocessing eliminates systematic location and dispersion differences among heterogeneous variables, giving all of them the same *a priori* importance (mean values equal to 0 and standard deviations equal to 1), and enhances differences among the samples. Also, in the case of signals like current/potential profiles, in which all the variables have the same nature and measurement unit, column autoscaling may be important if there are variables characterized by a relatively low mean value and/or standard deviation, which enclose useful information. Otherwise, this pretreatment may decrease the signal-to-noise ratio, since the same weight is given both to the noisy parts of the signal and to the informative features.

3. Signal compression and variable reduction

Not rarely, analytical signals are composed of hundreds or thousands of variables. Many of them are uniquely or predominantly associated with noise, while contiguous informative variables are often very intercorrelated, so that they carry redundant information. For these reasons, chemometric tools to perform the selection of a limited number of informative predictors and to realize data compression are enormously profitable.

A reliable way to compress a set of signals is to perform principal component analysis (PCA) (see Section VI.C) and then use a limited number of significant principal components (PCs) as new variables to describe the samples.

a. Wavelet transform The wavelet transform (WT) is a technique developed to study the frequency components of a signal, similar to the Fourier transform, with the main aim of compressing data.

The WT is based on the repeated application of low-pass filters (whose outputs are called approximations) and high-pass filters (whose outputs are called details) to a signal. There are many types of filters, which constitute the families of wavelets. The simplest is the Haar filter, based on semisums and semidifferences of consecutive signal elements (Haar, 1910). The discrete wavelet transform (DWT) scheme computes, in each level, approximations and details of the approximations of the previous level, originating the so-called Mallat pyramid. Instead, the wavelet packet transform (WPT) applies the filters also to the details at each level, producing a more extended tree. A set of wavelet coefficients (approximations and details) from which it is possible to reconstruct perfectly the original signal is called basis. Both for DWT and WPT, it is possible to define a number of different coefficient combinations, that is, different possible bases; in general, WPT allows more alternatives than DWT. The so-called best basis is the one, among all possible wavelet bases, which optimizes a given criterion function, a cost function, like threshold or entropy (Mallet *et al.*, 2000; Soman and Ramachandran,

2005). Usually, several coefficients are very small, so that they can be omitted without a significant negative effect on the reconstructed signal and, hopefully, with the elimination of noise. The result is the compression of the signal.

Generally, chemometrics handles data sets constituted by many objects described by the same variables. In this perspective, the application of wavelet transform should be performed, obtaining, for all the objects, a single basis formed by the same coefficients, the so-called common best basis.

Usual procedures for the selection of the common best basis are based on maximum variance criteria (Walczak and Massart, 2000). For instance, the variance spectrum procedure computes at first the variance of all the variables and arranges them into a vector, which has the significance of a spectrum of the variance. The wavelet decomposition is applied onto this vector and the best basis obtained is used to transform and to compress all the objects. Instead, the variance tree procedure applies the wavelet decomposition to all of the objects, obtaining a wavelet tree for each of them. Then, the variance of each coefficient, approximation or detail, is computed, and the variance values are structured into a tree of variances. The best basis derived from this tree is used to transform and to compress all the objects.

b. SELECT: A decorrelation–selection algorithm As already discussed in the case for almost continuous signals such as voltammograms, not all variables contribute unique or useful information. The elimination of predictors of limited or negligible utility—noisy or redundant—may increase the efficiency of models and/or make their interpretation simpler. Stepwise decorrelation of variables was introduced by B. Kowalski and C.F. Bender as an algorithm with the name SELECT (Kowalski and Bender, 1976), a supervised Gram–Schmidt orthogonalization. This algorithm can be used both for classification/class-modeling problems and multivariate quantitative calibration. It is based on a sequential identification and decorrelation of individual variables. For example, SELECT first identifies the variable with the largest discriminant power (e.g., the Fisher weight) in the case of classification and class-modeling problems. This first selected variable is decorrelated from all the others so that these latter ones become orthogonal to that selected. After decorrelation, the selected variable is set aside from the variable set and the process repeated with another identification. This identification and decorrelation procedure continues until the decorrelated predictors reach a discriminant power value less than a prefixed cut-off level. In the case of regression, the selection criterion is based on the correlation coefficient of predictors with a given response variable.

C. Exploratory analysis

1. Principal component analysis

PCA is one of the most widely employed and most useful tools in the branch of exploratory analysis. It has been used in more than half of the 230 electronic tongue studies examined for this review, as shown in Fig. 2.10. It offers a general overview of the problem studied, showing the interrelations existing among objects and between objects and variables. Also intercorrelations among variables can be estimated, and this knowledge may be used subsequently, for instance, for eliminating redundant information.

Since a high variability (i.e., a high variance value) is synonymous with a high amount of information (see Fig. 2.1), the PCA algorithms search for the maximum variance direction, in the multidimensional space of the original data, preferably passing through the data centroid, which means that data have to be at least column-centered. The maximum variance direction, which can be expressed as a linear combination of the original variables, represents the first PC . The second PC is the direction which keeps the maximum variance among all directions orthogonal to the first PC. Therefore, the second PC explains the maximum information not explained by the first one or, in other words, these two new variables

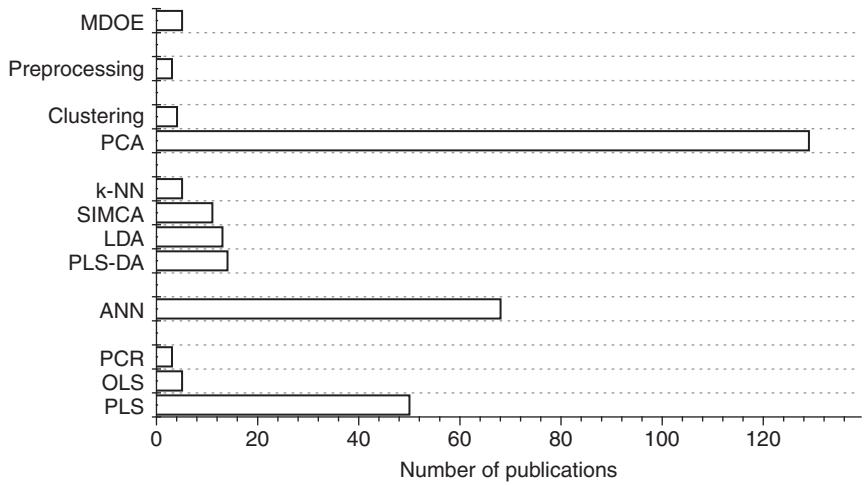

FIGURE 2.10 Main chemometric techniques applied in electronic tongue studies. Data obtained from a literature search over the period 1996–2009, using SciFinder Scholar. MDOE, multivariate design of experiments; PCA, principal component analysis; k-NN, *k*-nearest neighbors; SIMCA, soft independent modeling of class analogy; LDA, linear discriminant analysis; PLS-DA, partial least squares discriminant analysis; ANN, artificial neural networks; PCR, principal component regression; OLS, ordinary least squares; PLS, partial least squares.

are not intercorrelated among them. The process goes on identifying the subsequent PCs: it may stop at reaching a variance cut-off value or continue until all the variability enclosed in the original data has been explained.

Since the variance values depend on the measurement unit of variables, it becomes difficult to compare and impossible to combine information from variables of different nature, unless properly normalized: column autoscaling is the transform most commonly applied.

Each object can be projected in the space defined by the new variables: the coordinate values obtained are called scores.

As already stated, the PCs are expressible as linear combinations of the original variables: the coefficients which multiply the variables are called loadings. They represent the cosine values (director cosines) of the angles between the PCs and the original variables. These values may vary between -1 and $+1$, indicating the importance of defining a given PC: the larger the cosine absolute value, the closer the two directions, thus the larger the contribution of the original variable to the PC.

In terms of matrix algebra, the rotation from the space of the original variables to the PC space is performed by means of the loading orthogonal matrix, **L**:

$$\mathbf{S}_{\mathrm{NV}} = \mathbf{X}_{\mathrm{NV}}\mathbf{L}_{\mathrm{VV}} \tag{6}$$

where **S** is the score matrix and **X** is the original matrix, constituted by N objects (rows) described by V variables (columns).

The key feature of PCA is in its high capability for representing large amounts of complex information by way of simple bidimensional or tridimensional plots.

In fact, the space described by two or three PCs can be used to represent the objects (score plot), the original variables (loading plot), or both objects and variables (biplot). For instance, if the first two PCs (low-order) are drawn as axes of a Cartesian plane, we may observe in this plane a fraction of the information enclosed in the original multidimensional space which corresponds to the sum of the variance values explained by the two PCs. Since PCs are not intercorrelated variables, no duplicate information is shown in PC plots.

In Fig. 2.11, an example of a biplot is shown (Rudnitskaya *et al*. 2009b). From the inspection of the graph, it is possible to get information about the repeatability of the measurements (replicate analyses on wine samples), the discrimination among samples (wines of three vintages), the intercorrelation between variables (responses of potentiometric sensors), and their discriminatory importance.

In the case of almost continuous signals such as voltammograms, it may be useful to represent the loading profiles, eventually superimposed

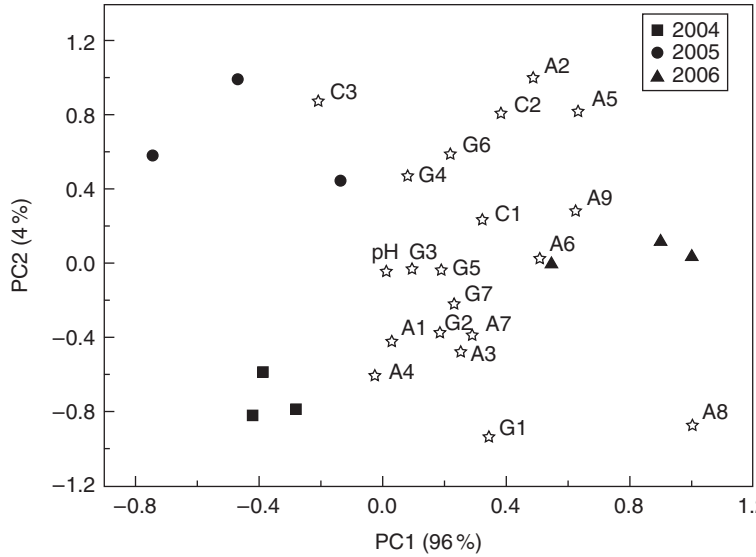

FIGURE 2.11 Example of biplot. The scores (filled symbols) are replicates of analyses of wine samples of three vintages (2004, 2005, and 2006, respectively), while the loadings (stars) represent the potentiometric sensors used for the measurements (A = anion-sensitive sensors, C = cation-sensitive sensors, G = redox-sensitive sensors, pH = pH sensor) (reproduced from Rudnitskaya *et al.* 2009b, with permission).

to a selected signal or an average signal profile, in order to directly visualize which parts of the original signals give the highest contribution in defining a particular PC. As already remarked, particular caution in interpretations has to be observed when a row normalization such as the SNV transform has been applied to the signals. In fact, such a transform might shift or smear the information contained in one region of the original signals (Fearn, 2009).

As already stated, maximum variance means maximum information. Nevertheless, it has to be noted that the maximum information does not always correspond to the most useful information for the solution to a particular problem. In fact, there are cases in which the maximum variability characterizing a data set refers to factors that are not related to the features of interest. In such cases, the useful information might be extracted by higher-order PCs, explaining a minor (but, in this case, effective) variance fraction (Jolliffe, 2002). For example, if the aim of a study is the discrimination of wine samples on the basis of the oenological production region and the samples have been collected spanning three vintages, the highest variability in the data, thus captured by the low-order PCs, will very probably be year-related. On the other hand, an

examination of the scores on higher-order PCs may show groupings due to sample provenance.

PCA is a useful tool not only for visualizing information; it can also be employed as a strategy for feature reduction and noise reduction, as indicated in Section VI.C.

2. Clustering

Clustering is a branch of exploratory analysis able to provide answers about the presence of groupings among objects or variables, by means of a similarity measurement (Vandeginste *et al.*, 1998). The similarity among two objects is defined as an inverse function of their distance: the more two objects are distant, the less they are similar. Several metrics may be used to evaluate the distance D between two objects i and j in a n-dimensional space. The most common are

$$\text{Euclidean} : D_{i,j} = \sqrt{\sum_{v=1}^{V} \left(x_{i,v} - x_{j,v}\right)^2} = \sqrt{\left(\mathbf{x}_i - \mathbf{x}_j\right)'\left(\mathbf{x}_i - \mathbf{x}_j\right)} \tag{7}$$

$$\text{Mahalanobis} : D_{i,j} = \sqrt{\left(\mathbf{x}_i - \mathbf{x}_j\right)'\mathbf{V}^{-1}\left(\mathbf{x}_i - \mathbf{x}_j\right)} \tag{8}$$

In every case, the similarity value S between the objects i and j is computed as

$$S_{i,j} = 1 - \frac{D_{i,j}}{D_{\max}} \tag{9}$$

where $D_{\max}$ is the maximum distance between all the possible pairs of objects in the data set. It follows that the values of the parameter S may vary between two limits: 0 when the two objects considered are those with maximum interdistance and 1 if the two objects are coincident.

There are two main types of clustering techniques: hierarchical and nonhierarchical. Hierarchical cluster analysis may follow either an agglomerative or a divisive scheme: agglomerative techniques start with as many clusters as objects and, by means of repeated similarity-based fusion steps, they reach a final situation with a unique cluster containing all of the objects. Divisive methods follow exactly the opposite procedure: they start from an all-inclusive cluster and then perform a number of consecutive partitions until there is a bijective correspondence between clusters and objects (see Fig. 2.12). In both cases, the number of clusters is defined by the similarity level selected.

The results can be visualized in a plot called a dendrogram, in which the similarity or distance values corresponding to each fusion/partition step are represented. One of the two axes corresponds to the similarity,

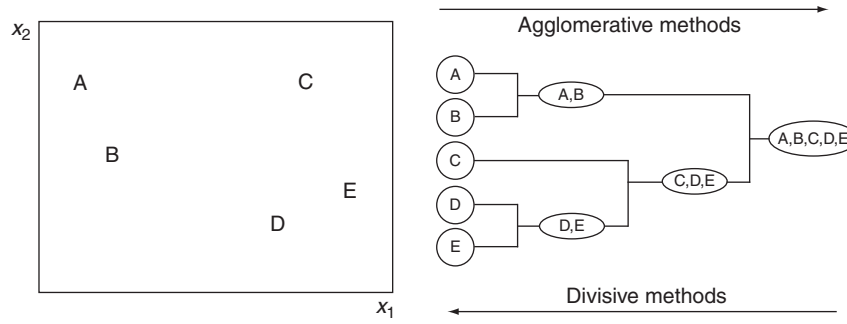

FIGURE 2.12 Scheme of agglomerative and divisive clustering approaches illustrated with five objects described by two variables.

while the second one reports the objects following an arbitrary order without any physical implication. Such plots may be very useful for identifying the similarity cut-off level and thus the number of clusters.

D. Classification and class-modeling

Classification and class-modeling techniques may provide qualitative answers to many problems of interest in the field of electronic tongue-based studies. They build mathematical rules or models able to characterize a sample with respect to a qualitative property, that is the class to which it belongs. For example, in the case of food problems, they may be useful in order to determine whether a product is genuine or adulterated, whether its quality is acceptable or poor, the identification of its geographical origin, the identity of the cultivar of vegetable ingredients used, which manufacturing technologies have been employed, and so on. In fact, a class (or category) is defined as a grouping of samples characterized by the same value of discrete variables or by contiguous values of continuous variables. Frequently, such variables are qualitative factors whose values cannot be determined experimentally, so that they have to be estimated from the values of some experimentally measurable predictors, by way of suitable mathematical tools.

More in detail, classification techniques are able to determine to which class a sample more probably belongs, among a number of predefined classes. They work by building a delimiter between the classes. Then, each new object is always assigned to the category to which it more probably belongs, even in the case of objects which are not pertinent to any class studied.

Instead, class-modeling techniques verify whether a sample is compatible or not with the characteristics of a given class of interest. In fact,

they provide an answer to the general question: ''Is sample X, claimed to belong to class A, really compatible with the class A model?''. This is essentially the question to be answered in most of the real qualitative problems studied by means of electronic tongues. In addition, such an approach is capable of detecting outliers.

Nevertheless, in most of the electronic tongue applications found in the literature, classification techniques like linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA) have been used in place of more appropriate class-modeling methods. Moreover, in the few cases in which a class-modeling technique such as soft independent modeling of class analogy (SIMCA) is applied, attention is frequently focused only on its classification performance (e.g., correct classification rate). Use of such a restricted focus considerably underutilizes the significant characteristics of the class-modeling approach.

A class model is characterized by two distinctive parameters: sensitivity and specificity. Sensitivity is defined as the percentage of objects belonging to the modeled class which are rightly accepted by the model. Specificity is the percentage of objects not belonging to the modeled class which are rightly rejected by the model. A class-modeling technique builds a class space around a mathematical class model, with a wideness corresponding to the confidence interval, at a preselected confidence level, for the class objects: sensitivity is an experimental measure of this confidence level. A decrease in the confidence level for the modeled class generally reduces the sensitivity and increases the specificity of the model. Frequently, in order to evaluate the model performance, taking into account both these features, an efficiency parameter is computed as the mean (better, the geometric mean) of the sensitivity and specificity values.

The results of class-modeling analyses can be visualized by way of the Coomans' plots (Coomans *et al.*, 1984) which, as in the example of Fig. 2.13, represent the objects of the data set studied in relation to the models of two classes, A and B. The two Cartesian axes correspond to the distances from the class A model and from the class B model, respectively, while two straight lines, parallel to the axes, describe the corresponding class spaces at the predetermined confidence level. The plot area is divided into four regions, which contain respectively: the objects accepted by the class A model (upper left rectangle), the objects accepted by the class B model (lower right rectangle), the objects accepted by both the models (lower left square), and the objects rejected by both the models (upper right square).

Classification and class-modeling techniques belong to three main families:

- distance-based techniques
- probabilistic techniques
- experience-based techniques.

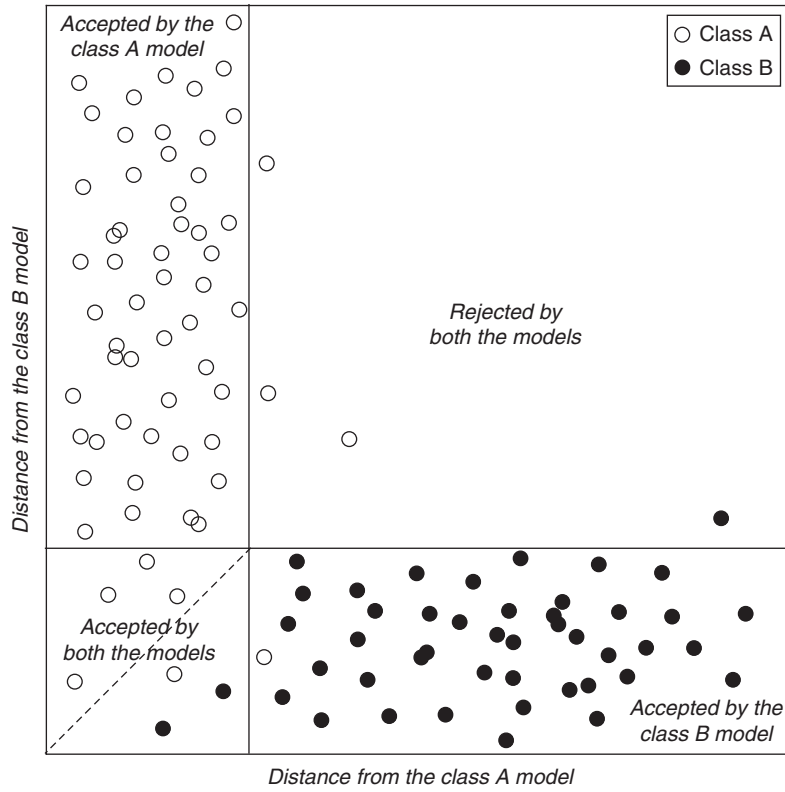

FIGURE 2.13 Example of Coomans' plot.

1. *k*-Nearest neighbors

k-Nearest neighbors (k-NN) is one of the simplest approaches for classification. It is a distance-based technique, which predicts the class membership of a sample on the basis of the class of the *k* sample(s) nearest to it in the multidimensional space (Vandeginste *et al.*, 1998).

To classify a new sample, *k*-NN computes its distances (usually, the multivariate Euclidean distances, see Eq. 7) from each of the samples of a training set, whose class membership is known. The *k* nearest samples are then taken into consideration to perform the classification: generally, a majority vote is employed, meaning that the new object is classified into the class mostly represented within the *k* selected objects. Being a distance-based method, it is sensitive to the measurement units and to the scaling procedures applied.

The method provides a delimiter between categories, which is typically nonlinear, generally expressible as a piecewise linear function (see Fig. 2.14). The delimiter usually becomes more smoothed for elevated

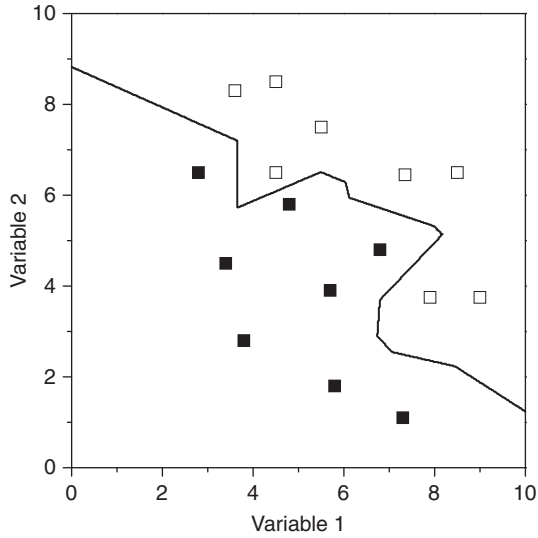

FIGURE 2.14 Example of *k*-NN class delimiter for $k = 1$.

values of *k*. When the parameter *k* is optimized to obtain the highest prediction ability for a given data set, validation should be performed by way of a three-set procedure (see Section VI.F).

k-NN has been shown to perform as well as or better than more complex methods in many applications (Dudoit *et al.*, 2002; Vandeginste *et al.*, 1998). Furthermore, being a nonparametric method, it is free from statistical assumptions such as normality of variable distributions, so that its applicability is much wider than that of parametric probability-based techniques.

2. Linear discriminant analysis

LDA is the first classification technique introduced into multivariate analysis by Fisher (1936). It is a probabilistic parametric technique, that is, it is based on the estimation of multivariate probability density functions, which are entirely described by a minimum number of parameters: means, variances, and covariances, like in the case of the well-known univariate normal distribution. LDA is based on the hypotheses that the probability density distributions are multivariate normal and that the dispersion is the same for all the categories. This means that the variance–covariance matrix is the same for all of the categories, while the centroids are different (different location). In the case of two variables, the probability density function is bell-shaped and its elliptic section lines correspond to equal probability density values and to the same Mahalanobis distance from the centroid (see Fig. 2.15A).

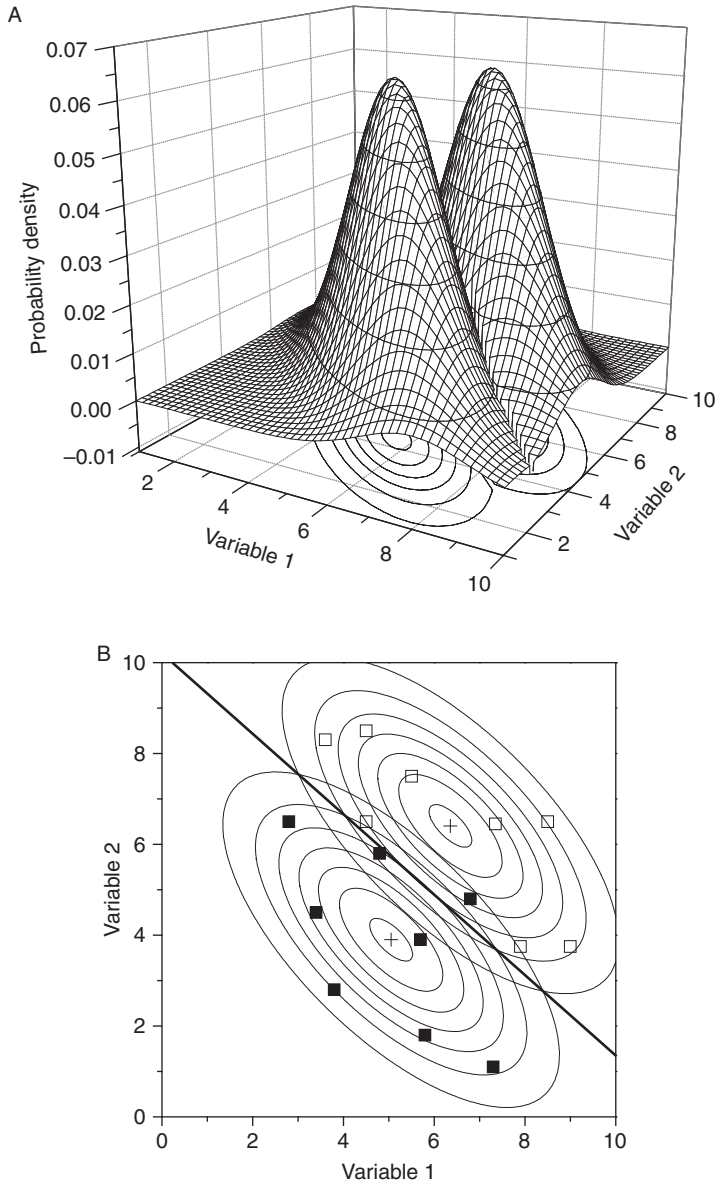

FIGURE 2.15 Bivariate probability distributions (A), iso-probability ellipses and LDA delimiter (B).

The iso-probability region described by the equation of the Mahalanobis distance is an ellipsoid in the case of three variables or a hyper-ellipsoid in the case of more than three variables.

The Mahalanobis distance can be considered as an Euclidean distance modified for taking into account the dispersion and the intercorrelation of all of the variables (see Eqs. 7 and 8).

Because of the aforementioned LDA hypotheses, the ellipses of different categories present equal eccentricity and axis orientation: they only differ for their location in the plane. By connecting the intersection points of each couple of corresponding ellipses, a straight line is identified which corresponds to the delimiter between the two classes (see Fig. 2.15B). For this reason, this technique is called linear discriminant analysis. The directions which maximize the separation between classes are called LDA canonical variables.

3. Quadratic discriminant analysis

Quadratic discriminant analysis (QDA) is a probabilistic parametric classification technique which represents an evolution of LDA for nonlinear class separations. Also QDA, like LDA, is based on the hypothesis that the probability density distributions are multivariate normal but, in this case, the dispersion is not the same for all of the categories. It follows that the categories differ for the position of their centroid and also for the variance–covariance matrix (different location and dispersion), as it is represented in Fig. 2.16A. Consequently, the ellipses of different categories differ not only for their position in the plane but also for eccentricity and axis orientation (Geisser, 1964). By connecting the intersection points of each couple of corresponding ellipses (at the same Mahalanobis distance from the respective centroids), a parabolic delimiter is identified (see Fig. 2.16B). The name quadratic discriminant analysis is derived from this feature.

4. Unequal class models

Unequal class models (UNEQ) is a powerful class-modeling technique, which originated in the work of H. Hotelling (Hotelling, 1947) and was introduced into chemometrics by M.P. Derde and D.L. Massart (Derde and Massart, 1986). This technique, derived from QDA, is based as well on the hypothesis of a multivariate normal distribution in each category studied and on the use of Hotelling's T^2 statistics to define a class space, whose boundary is an ellipse (two variables), an ellipsoid (three variables), or a hyper-ellipsoid (more than three variables). The dispersion of a class space is defined by the critical value of the T^2 statistics at a selected confidence level. The eccentricity and the orientation of the ellipse depend on the correlation between the variables and on their dispersion.

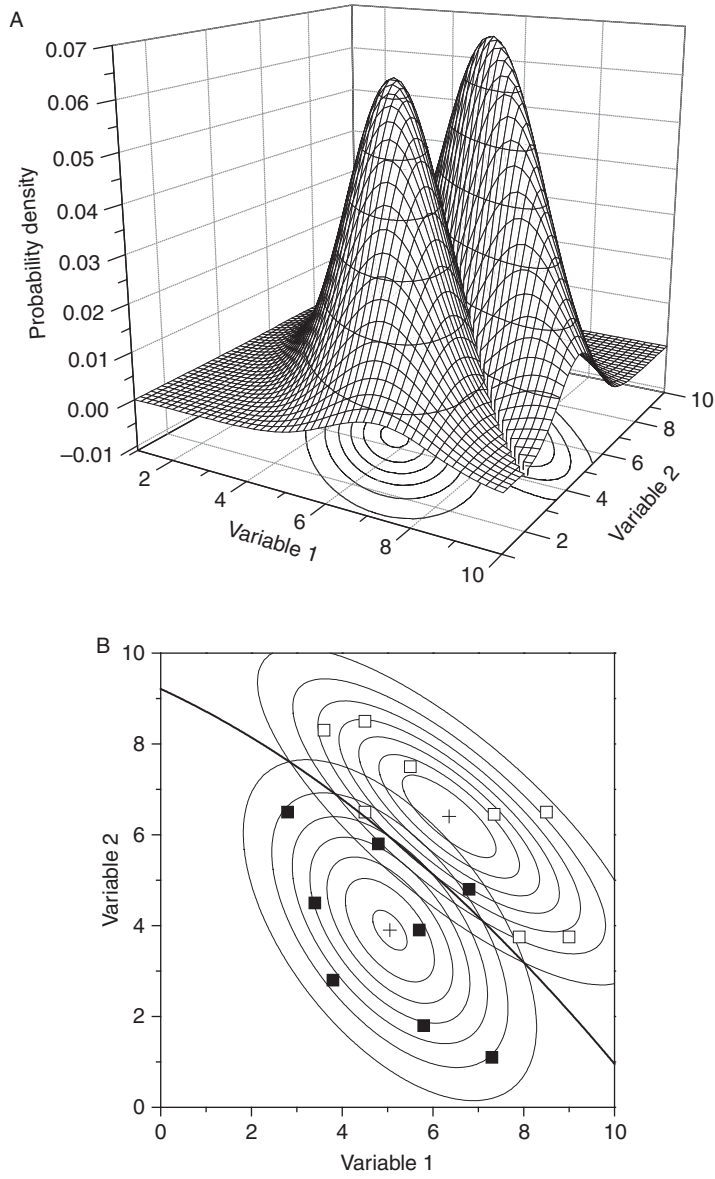

FIGURE 2.16 Bivariate probability distributions (A), iso-probability ellipses and QDA delimiter (B).

LDA and QDA-UNEQ present some restrictions on the number of objects that can be used. From a strictly mathematical point of view, objects have to be one more than the number of variables measured. Nevertheless, in order to obtain reliable results, these techniques should be applied in cases when the ratio between the number of objects in a given category and the number of the variables is at least three. Furthermore, the number of objects in each class should be nearly balanced: it is not advisable to work when ratios between number of objects in different categories are greater than three.

In cases involving many variables (such as voltammetric data), it is possible to apply LDA and QDA-UNEQ following a preliminary reduction in the variable number, for instance, by PCA or wavelet compression.

5. Soft independent modeling of class analogy

SIMCA (Wold and Sjöström, 1977) was the first class-modeling technique used in chemometrics. Being a modeling technique, each class is modeled independently of the others. The central feature of this method is the application of PCA to the sample category studied, generally after within-class autoscaling or centering. SIMCA models are defined by the range of the sample scores on a selected number of low-order PCs—ideally the significant ones—and models therefore correspond to rectangles (two PCs), parallelepipeds (three PCs), or hyper-parallelepipeds (more than three PCs) referred to as the multidimensional boxes of SIMCA inner space. Conversely, the PCs not used to describe the model define the outer space, considered as uninformative space. The score range can be enlarged or reduced, mainly depending on the number of samples, to avoid the possibility of under- or overestimation of the true variability (Forina and Lanteri, 1984). The standard deviation of the distance of the objects in the training set from the model corresponds to the class standard deviation. The boundaries of SIMCA space around the model are determined by a critical distance, which is obtained by means of Fisher statistics, so that the shape of the class space is not exactly that of a parallelepiped. There is no specific hypothesis other than that this distance should be normally distributed. However, the distribution of samples in the inner space should be more or less uniform; otherwise, regions in the inner space lacking objects from the modeled class would be incorrectly considered as a part of the model.

SIMCA is a very flexible technique since it allows variation in a large number of parameters such as scaling or weighting of the original variables, number of components, expanded or contracted score range, and confidence level applied.

Furthermore, SIMCA computes a number of useful parameters, such as the modeling power of the variables (their contribution to the inner space), the discriminating power of the variables (when more than one

category is studied), and the distance between the categories. Variables with both small modeling and discriminating power may be eliminated, in order to obtain refined models.

6. Artificial neural networks

Artificial neural networks (ANNs) have been widely applied in the electronic tongue literature both for classification and multivariate regression problems: almost one-third of the papers on electronic tongues examined for this review show ANN applications (see Fig. 2.10).

ANNs offer some advantages. For instance, they are generally well suited for nonlinear problems, and the related software is easily available. However, a number of important drawbacks should limit ANN use only to the cases in which other techniques fail and a great number of samples are available.

Multilayer feed-forward neural networks (MLF) represent the type of ANNs most widely applied to electronic tongue data. Their scheme is shown in Fig. 2.17.

MLF are composed of a number of computational elements, called neurons, generally organized in three layers (Marini, 2009). In the first one, the input layer, there are usually N neurons which correspond to the original predictor. The predictors are scaled (generally range scaled). When the number of original predictors is very high, the PCs may be used, in order to reduce the data amount and the computational time.

The first layer transmits the value of the predictors to the second—hidden—layer. All the neurons of the input layer are connected to the J neurons of the second layer by means of weight coefficients, meaning that the J elements of the hidden layer receive, as information, a weighted sum S of the values from the input layer. They transform the information received (S) by means of a suitable transfer function, frequently a sigmoid.

These neurons transmit information to the third—output—layer, as a weighted combination (Z) of values. The neurons in the output layer correspond to the response variables which, in the case of classification, are the coded class indices. The output neurons transform the information Z, from the hidden layer, by means of a further sigmoid function or a semilinear function.

After a first random initialization of the values, a learning procedure modifies the weights $w_{n,j}$ and w_j during several optimization cycles, in order to improve the performances of the net. The correction of the weights at each step is proportional to the prediction error of the previous cycle. The optimization of many parameters and the elevated number of learning cycles considerably increase the risk of overfitting and, for this reason, a deep validation is required, with a consistent number of objects.

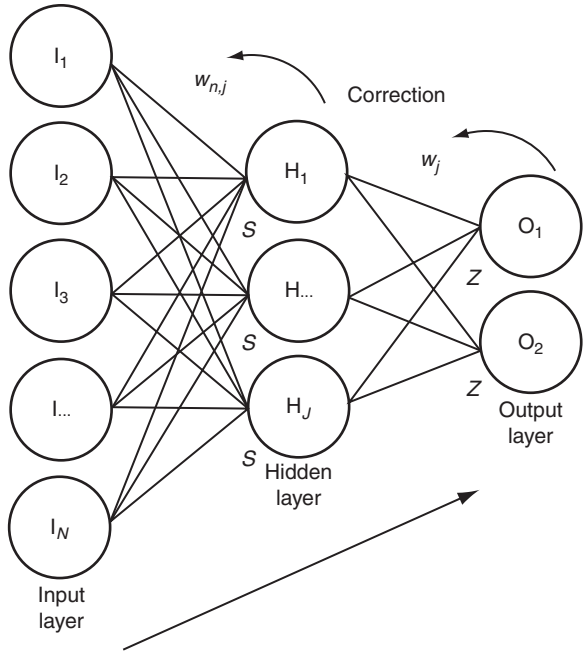

FIGURE 2.17 Scheme of multilayer feed-forward neural networks.

Another type of ANNs widely employed is represented by the Kohonen self organizing maps (SOMs), used for unsupervised exploratory analysis, and by the counterpropagation (CP) neural networks, used for nonlinear regression and classification (Marini, 2009). Also, these tools require a considerable number of objects to build reliable models and a severe validation.

Nevertheless, in many electronic tongue studies, such constraints are ignored and ANNs are used as the default choice. This choice is also made in cases with very poor data sets and without performing a proper validation. This may be due to the fact that the related computational software is easily available and that many people have a propensity to follow the predominant trends and to use the most potent instruments available, without critical considerations. Furthermore, perhaps, there is a fashionable association of ideas connecting the concepts of artificial tongue and artificial intelligence.

7. State-of-the-art class-modeling techniques

Two class-modeling techniques have recently been introduced: multivariate range modeling (MRM) and CAIMAN analogues modelling methods (CAMM).

MRM follows a simple and intuitive way of building class models by employing the ranges of the predictors (Forina *et al.*, 2008). In order to take into account intercorrelations in the data set, several new variables are computed as linear combinations of the original predictors and employed to build the models: LDA canonical variables represent a typical example. A basic feature of MRM is the capability of providing, by definition, class models with 100% sensitivity: this property is very important when a model is built for a food protection consortium, the production of whose affiliated producers must all be recognized and accepted by the model. In addition, the eventual presence of noninformative variables does not have a negative influence on the model specificity. A further advantage of MRM can be found in its outcome, which is easily understandable and interpretable also by people with a limited knowledge of multivariate analysis.

CAMM is a family of powerful class-modeling techniques which builds models using distances (leverages or Mahalanobis distances from the class centroids) as predictors (Forina *et al.*, 2009). Such new variables may be used separately or in combination with the original predictors and, in many cases, supply models characterized by excellent efficiency values.

E. Regression

Regression techniques provide models for quantitative predictions. The ordinary least squares (OLS) method is probably the most used and studied historically. Nevertheless, it presents a number of restrictions which often limit its applicability in the case of artificial tongue data.

1. Ordinary least squares

OLS regression, also known as multivariate linear regression (MLR) (Draper and Smith, 1981), searches for the linear function corresponding to the smallest value of the sum of the squared residuals:

$$\sum_{i=1}^{I} \left(y_i - \hat{y}_i\right)^2 \tag{10}$$

where y_i is the actual value of the response variable for the object i, and $\hat{y}_i$ is the value computed by the model, which can be expressed as a mathematical relationship between response and predictors:

$$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + \cdots + b_V x_V \tag{11}$$

that is, in the matrix notation:

$$\hat{\mathbf{y}} = \mathbf{X}'\mathbf{b} \tag{12}$$

X is the matrix of the predictors augmented with a column of 1, necessary for the estimation of the intercept values. **b** is the column vector of the regression coefficients. The regression coefficients are estimated by

$$\mathbf{b} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y} \tag{13}$$

The elements of the vector y are the reference values of the response variable, used for building the model. The uncertainty on the coefficient estimation varies inversely with the determinant of the information matrix ($\mathbf{X}'\mathbf{X}$) which, in the case of a unique predictor, corresponds to its variance. In multivariate cases, the determinant value depends on the variance of the predictors and on their intercorrelation: a high correlation gives a small determinant of the information matrix, which means a big uncertainty on the coefficients, that is, unreliable regression results.

Unfortunately, electronic tongue variables are very often considerably intercorrelated: in voltammetric profiles, for instance, currents evaluated at two consecutive potential values frequently carry almost the same information, so that their correlation coefficient is nearly 1. In such cases, standard OLS is absolutely not recommendable. Furthermore, the number of objects required for OLS regression must be at least equal to the number of predictors plus 1, and it is difficult to satisfy such a condition in many practical cases.

2. Principal component regression

A very simple strategy to overcome these hurdles is offered by PCA: the PCs may be used as new predictors because they are, by definition, orthogonal, that is, uncorrelated. The response is calculated as a function of a small number of significant PCs, computed from the original variables (Jolliffe, 1982). This technique, which is very efficient in many cases, is known as principal component regression (PCR). Since the directions that explain the highest variance amount (i.e., the lowest-order PCs) are not always the most important in predicting a response variable, it is possible to follow a refined approach, which performs a step-wise selection of the PCs to be used in the model on the basis of their modeling efficiency.

3. Partial least squares

A further better solution is offered by partial least squares (PLS), whose acronym may signify also projections onto latent structures. These latent structures, more frequently called latent variables (LVs) or PLS components, are directions in the space of the predictors with a connotation

similar to PCs: while the first PC is the maximum variance direction, the first latent variable is the direction characterized by the maximum covariance with the selected response variable.

The information related to the first latent variable is then subtracted from both the original predictors and the response. The second latent variable is orthogonal to the first one, being the direction of maximum covariance between the residuals of the predictors and the residuals of the response. This approach continues for additional LVs.

The optimal complexity of the PLS model, that is, the most appropriate number of latent variables, is determined by evaluating, with a proper validation strategy (see Section VI.F), the prediction error corresponding to models with increasing complexity. The parameter considered is usually the standard deviation of the error of calibration (SDEC), if computed with the objects used for building the model, or the standard deviation of the error of prediction (SDEP), if computed with objects not used for building the model (see Section VI.F).

$$\mathrm{SDEC}(P) = \sqrt{\frac{\sum_{i=1}^{N} \left(y_i - \hat{y}_i\right)^2}{N}} \tag{14}$$

where y_i is the value of the response variable y for object i, $\hat{y}_i$ the corresponding value computed or predicted by the model, and N is the number of objects used for computing the parameter.

In general, the calibration error always decreases when the number of LVs augments, because the fitting increases (toward an overfitting). On the contrary, the prediction error generally decreases until a certain model complexity and then raises: this indicates that the further LVs being introduced are bringing noise, as shown in Fig. 2.18. A simple and practical criterion is the choice of the LV number corresponding to the SDEP absolute minimum or—better—to the first local minimum as the optimal model complexity.

When the number of noisy (noninformative) variables is too large, PLS models may also supply rather poor predictive performance. In order to overcome such a matter, a number of techniques for the elimination of noisy variables or the selection of useful predictors have been deployed, such as iterative stepwise elimination (ISE), iterative predictor weighting (IPW), uninformative variable elimination (UVE), and Martens uncertainty test (MUT) (Forina *et al.*, 2007).

A number of PLS variants have been deployed, for instance, for developing nonlinear models and for predicting together several response variables (PLS-2). Furthermore, when category indices are taken as response variables, PLS may work as a classification method which is usually called PLS discriminant analysis (PLS-DA).

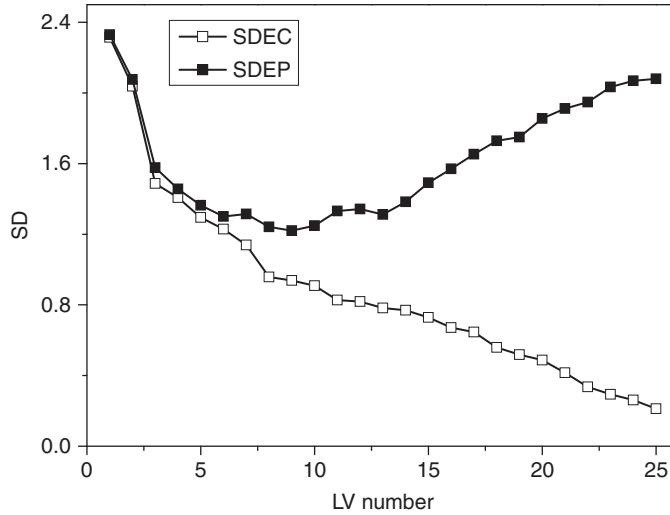

FIGURE 2.18 Typical profile of calibration and prediction errors as a function of the PLS model complexity (number of latent variables). The examination of such a plot may be helpful in selecting the optimal model complexity.

F. Validation

As claimed in Section VI, models are false, but in some cases, they may be useful. In chemometrics, models are generally aimed at predicting a quantity or a property of interest: the usefulness of a model is thus measured by its reliability in prediction. Prediction ability values should be presented with their confidence interval (Forina *et al.*, 2001, 2007), which depends very much on the number of objects used for the validation. The estimation of predictive ability on new objects—not used for building the models—is a fundamental step in any modeling process, and several procedures have been deployed for this purpose. The most common validation strategies divide the available objects in two subsets: a training (or calibration) set used for calculating the model and an evaluation set used to assess its reliability. A key feature for an honest validation is that the test-objects have to be absolutely extraneous to the model: no information from them can be used in building the model, otherwise the prediction ability may be overestimated.

In many modeling techniques, the number of parameters is modified many times looking for a setting that provides the maximum predictive ability for the model. Techniques for variable selection and methods based on artificial neural networks perform an optimization, that is, they search for conditions able to provide the maximum predictive ability possible for a given sample subset.

A better validation strategy, in such cases, is to use three sample subsets: a training set, an optimization set, and an evaluation set. The optimization set is used to find the best modeling settings, while the actual reliability of the final model is estimated by way of a real prediction on the third subset, formed by objects that have never influenced the model. The three-set validation procedure should always be used in ANN modeling, which presents a very high risk of overfitting.

The evaluation of the predictive ability of a model can be performed in a unique step or many times with different evaluation sets, depending on the strategy adopted.

1. Single evaluation set

A single evaluation set is the simplest and most rapid validation scheme. A fraction—usually between 50% and 90% of the total number—of the available samples constitutes the training set, while the remaining objects form the evaluation set. The subdivision may be arbitrary, random, or performed by way of a uniform design, such as the Kennard and Stone and the duplex algorithm (Kennard and Stone, 1969; Snee, 1977), which allows two subsets to be obtained that are uniformly distributed and representative of the total sample variability.

2. Cross-validation

Cross-validation (CV) is probably the most common validation procedure. The N objects available are divided into G cancellation groups following a predetermined scheme (e.g., contiguous blocks, or Venetian blinds). The model is computed G times: each time, one of the cancellation groups is used as the evaluation set, while the other groups constitute the training set. At the end of the procedure, each object has been used G-1 times for building a model and once for evaluation. The number of cancellation groups usually ranges from 3 to N. Cross-validation with N cancellation groups is generally known as the leave-one-out procedure (LOO). LOO has the advantage of being unique for a given data set, whereas, when $G < N$, different orders of the objects and different subdivision schemes generally supply different outcomes. However, especially when the total number of objects is considerable, predictions made on a unique object, although repeated many times, may yield an overly optimistic result. An extensive evaluation strategy consists in performing cross-validation many times, with different numbers of cancellation groups, from 3 up to N. Another possibility is to repeat the validation, for a given number $G < N$ of cancellation groups, each time permuting the order of the objects, thus obtaining a different group composition each time.

3. Repeated evaluation set

This procedure, also called Monte Carlo validation, computes many models (not rarely 10,000–100,000), each time creating a different evaluation set, with an unfixed number of objects, by random selection. Each object may fall many times, or even no times at all, in the evaluation set. The main drawback of this validation strategy is the longer computational time.

VII. ARTIFICIAL TONGUE APPLICATIONS IN THE FOOD SCIENCE

Wine is the food which has been most extensively analyzed by means of electronic tongues. Typical qualitative studies concern the characterization of wine on the basis of vintage and vineyard.

Dos Santos *et al.* (2003) used impedance measurements to differentiate, by means of PCA, Cabernet wine samples on the basis of either the vintage or the vineyard. In another study by the same research group, the same data was processed with clustering techniques, in order to identify groupings, and with ANN classification, testing several algorithm parameter settings (Riul *et al.*, 2004).

Rodríguez-Méndez and coworkers applied an electronic tongue based on cyclic voltammetry and square wave voltammetry, with chemically modified electrodes, for discriminating via PCA, as shown in Fig. 2.19, a number of Spanish wines, of the same oenological region (Rioja) and vintage (2003), on the basis of the vineyard: Viura, Garnacha blanca, Tempranillo blanco, Malvasía, Turruntés (Rodriguez-Mendez *et al.*, 2008).

Buratti *et al.* (2004) employed an electronic tongue based on amperometric detection in a flow injection system (FIA), coupled with an electronic nose, to discriminate wines from vineyard Barbera produced in four Italian oenological regions with different denominations: Oltrepo' Pavese, Piemonte, Asti, and Alba. The chemometric techniques applied were PCA for data exploration, and LDA and CART (classification and regression trees) for classification.

Parra and coworkers used an electronic tongue based on cyclic voltammetry and square wave voltammetry to differentiate six Spanish red wines made from the same grape variety, prepared using a similar vinification method, but belonging to three different geographic origins (Rueda, Rioja, and Ribera del Duero) and different aging stages. The pattern recognition technique of PCA was applied (Parra *et al.*, 2004).

Also, Wu *et al.* (2005) proposed a method based on cyclic voltammetry for discriminating among six Chinese yellow wines of two brands (Kuaiji Mount, and Tower) on the basis of the aging, via PCA.

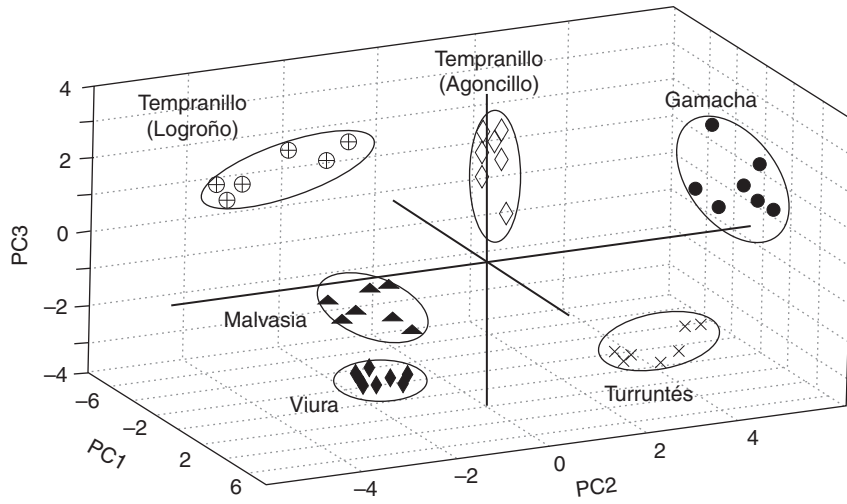

FIGURE 2.19 PCA 3d score plot (explained variance PC1 = 42%, PC2 = 21%, PC3 = 16%). Discrimination of Spanish red wines on the basis of the vineyard (reproduced from Rodriguez-Mendez *et al.*, 2008, with permission).

A similar study by Verrelli and coworkers, based on PCA of potentiometric data, allowed the differentiation of Verdicchio wines according to the winemaker and the vintage. Furthermore, MLR and PLS models, validated by means of cross-validation, permitted the quantification of a number of oenological parameters, such as SO_2, L-malic acid, and the total phenols content (Paolesse *et al.*, 2008; Verrelli *et al.*, 2007).

Parra *et al.* (2006a) developed a hybrid sensor array based on voltammetric electrodes, for monitoring the aging of red wines and for discriminating wine samples aged in oak barrels according to their characteristics (the wood origin and the toasting level). PCA score plots and SIMCA results revealed the high ability of the method proposed, as shown in Fig. 2.20, which represents the Coomans' plot for three-month and six-month aged wines, showing elevated sensitivity and specificity of the corresponding models.

In another work, Parra and coworkers proposed a method based on chemically modified voltammetric electrodes for the identification of adulterations made in wine samples, by addition of a number of forbidden adulterants frequently used in the wine industry to improve the organoleptic characteristics of wines, like, for example, tartaric acid, tannic acid, sucrose, and acetaldehyde (Parra *et al.*, 2006b). The patterns identified via PCA allowed an efficient detection of the wine samples that had been artificially modified. In the same study, PLS regression was applied for a quantitative prediction of the substances added. Model performances were evaluated by means of a cross-validation procedure.

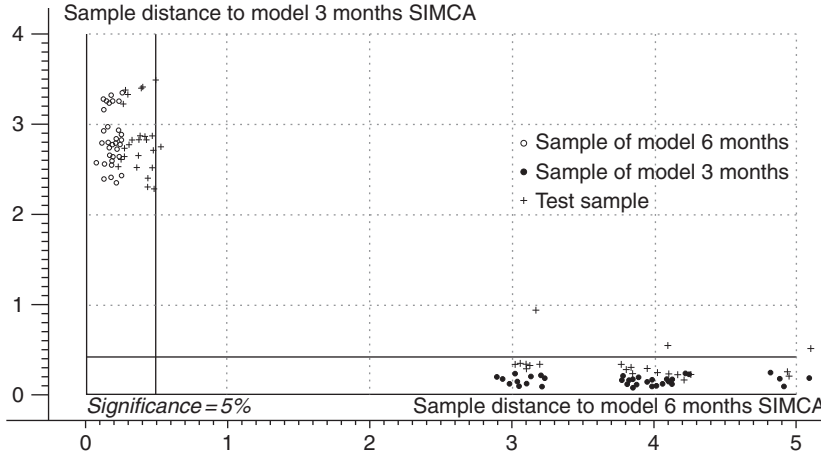

FIGURE 2.20 Coomans' plot of SIMCA models of three-months and six-months aged wine samples (reproduced from Parra *et al.*, 2006a, with permission).

Di Natale *et al.* (2000) used a potentiometric electronic tongue, compared with an electronic nose, for quantifying via ANN regression 23 parameters of oenological interest: density, effective alcohols, sugar, total alcohols, total extract, net extract, pH, total acids, volatile acids, total SO_2, ashes, alkalis, tartaric acid, malic acid, lactic acid, glycerol, polyphenols, antocyans, Ca, K, Mg, D_o 420 nm, and D_o 520 nm. The PC loading analysis revealed an evident distinction between electronic nose and electronic tongue, according to the assumption that the two systems provide independent information about the samples. Nevertheless, the ANN models presented cannot be considered valid, since the method was applied without a consistent number of samples and without a proper validation.

Other typical quantitative applications in the wine sector are related with the prediction of sensorial descriptors. Legin and coworkers used an array of 23 potentiometric sensors for predicting several sensorial attributes (visual, olfactory, and gustatory) in 56 wine samples by means of PLS and ANN regression models (Legin *et al.*, 2003). A test set validation was performed: a given fraction of all the samples was randomly chosen as the test set; all replicas of each sample were correctly included in the same set (calibration or test set). The models presented rather considerable prediction errors, especially for the determination of visual descriptors (nearly the 20%), as might be expected. For the prediction of gustatory attributes, the errors are about 5%, which is an acceptable result, considering that data from sensorial analysis are often characterized by comparable errors. In the same study, PLS models for the quantification of eight oenological parameters were built, obtaining lower prediction errors.

Buratti *et al.* (2006, 2007) employed an amperometric electronic tongue, together with a commercial electronic nose and spectrophotometric methods, to predict sensorial descriptors (total fruits, wood, spicy, sourness, bitterness, astringency, alcohol, body, overall quality) of Italian red dry wines of different denominations of origin. Genetic algorithms were employed to select the variables that maximized the predictive ability of the regression models; validation has been performed by means of a bootstrap procedure, closely related to the Montecarlo validation. Also in this case, the results are not very satisfactory (see Fig. 2.21), due to the intrinsic limits of data from sensorial analysis. As the same authors pointed out, ''Models with a good fitting performance (i.e., high R^2 value) do not always have an acceptable predictive performance.''

Rudnitskaya and coworkers developed PLS models for predicting the aging (from 10 to 35 years) of Port wine obtaining an error of about 1.5 years on the age estimation, which may be acceptable for practical applications. The samples were analyzed by means of an array of 28 potentiometric sensors. Data were preprocessed by OSC (orthogonal signal correction), a typical pretreatment for spectroscopic data; model validation was performed using one-third of the available samples as the external test set (Rudnitskaya *et al.*, 2007).

Labrador *et al.* (2009) developed a technique based on pulse voltammetry, used to predict concentrations of bisulfites, ascorbic acid, and histamine in wine samples, by means of PLS models evaluated via cross-validation. The best prediction results have been obtained for bisulfites.

In the first study describing a voltammetric electronic tongue, Winquist and coworkers presented a method for monitoring, by means of PC score projections of large amplitude and small amplitude pulse voltammetry data, the modifications of orange juice and milk after bottle opening during a 20-h period. The scores show a clear trend corresponding to the sampling time sequence and are different for the two foods: the orange juice presented considerable modifications at the beginning and, after about 8 h from bottle opening, reached a steady state. On the contrary, the milk showed a nearly constant trend during the first hours, while noticeable variations start to appear after 10 h (see Fig. 2.22). The authors explained such differences according to two different modification processes: a predominant oxidative alteration in the orange juice, a modest oxidation plus a gradual microbiological alteration in the milk (Winquist *et al.*, 1997). In further studies, the same authors improved the method, building PLS and ANN models for prediction of bacterial growth in milk samples (Winquist *et al.*, 1998). As a supplementary development, they presented a hybrid electronic tongue, based on a combination of pulse voltammetry, potentiometry, and conductivity measurements, able to distinguish, via PCA and ANN classification, six different types

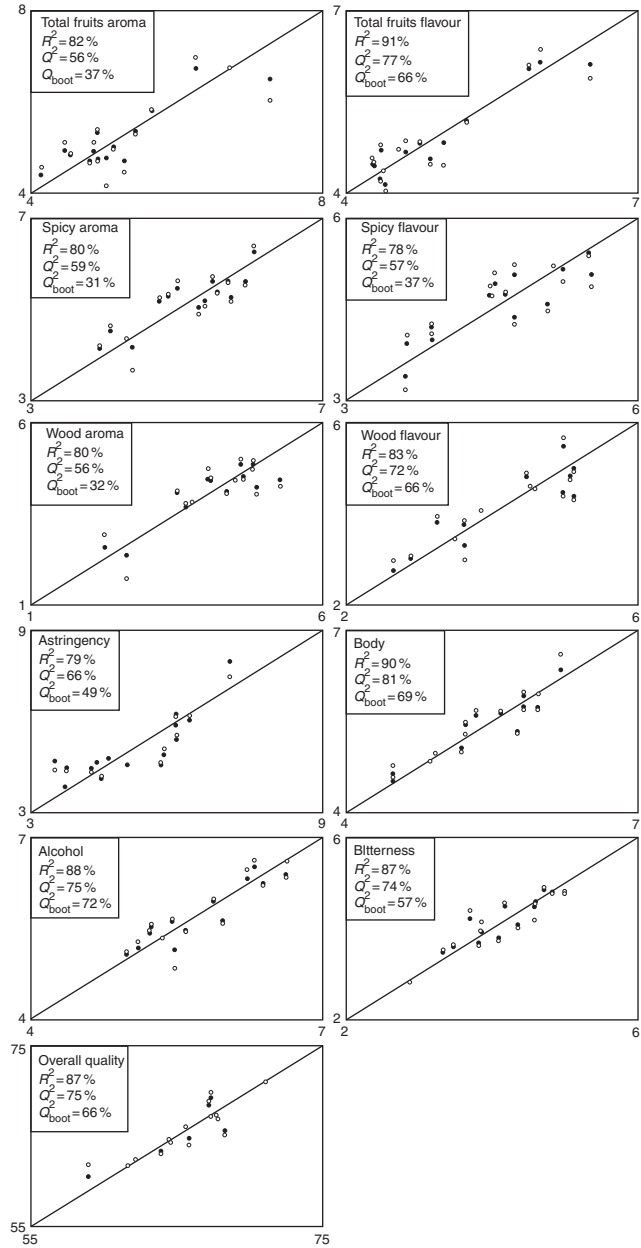

FIGURE 2.21 Scatter plot of the sensorial descriptors and overall quality estimated by e-nose, e-tongue, and spectrophotometric data. Plots show predicted (○) and calculated (•) versus experimental responses. The R^2, Q^2 leave-one-out, and Q^2 bootstrap values of each model are shown (reproduced from Buratti *et al.*, 2007, with permission).

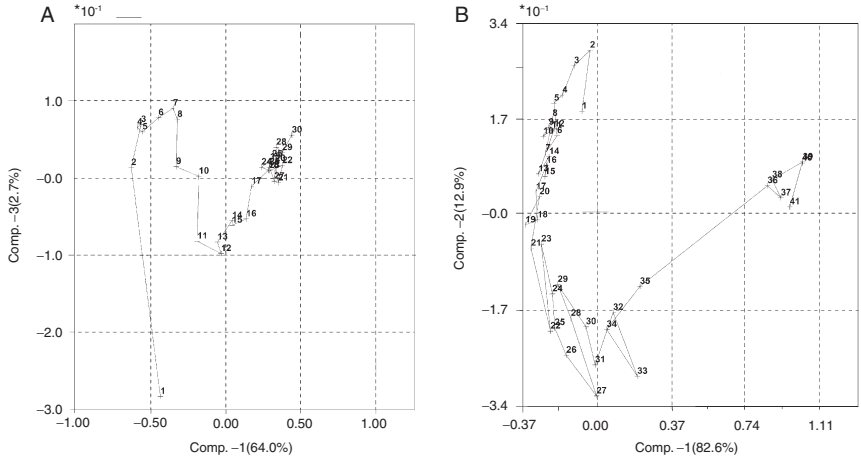

FIGURE 2.22 Aging processes of orange juice (A) and milk (B) monitored by PCA score plots from voltammetric data. The time between two consecutive samples is 30 min (reproduced from Winquist *et al.*, 1997, with permission).

of fermented milk: Filmjolk, A-fil, Kefir, Onaka, A-Yogurt, and Naturell Yogurt (Winquist *et al.*, 2000). In this study, model performances were evaluated on a test set constituted by only a sample for each class, with an elevated risk of overestimation of the actual predictive ability.

Artursson and Holmberg (2002) presented an approach for monitoring of water in drinking water production plants. The purpose of the study was to monitor the effect of different filters to determine how the filters work and when a filter starts to malfunction. The measurements are performed with a pulsed voltammetry-based electronic tongue. Data were preprocessed using a wavelet algorithm, with computation of the variance spectrum for the selection of the common best basis. The authors demonstrated that the groupings detectable in the PCA score plot correspond to changes in some chemical parameters (pH, alkalinity, and chemical oxygen demand (COD)), measured by reference instruments. The advantages of the electronic tongue presented in comparison with reference analyses are speed and the possibility of obtaining not only the detection of single analytes but also a qualitative overview of the water.

Lvova and coworkers used an electronic tongue composed of several potentiometric sensors and biosensors for distinguishing different types of tea and coffee and provide a quantification of compounds like tannic acid, caffeine, catechines, sugar, and L-arginine. PCA was applied for exploratory analysis, while SIMCA was used for building qualitative models (see the Coomans' plot in Fig. 2.23), and PCR and PLS were used for building quantitative models. The results presented are very satisfactory (Lvova *et al.*, 2003).

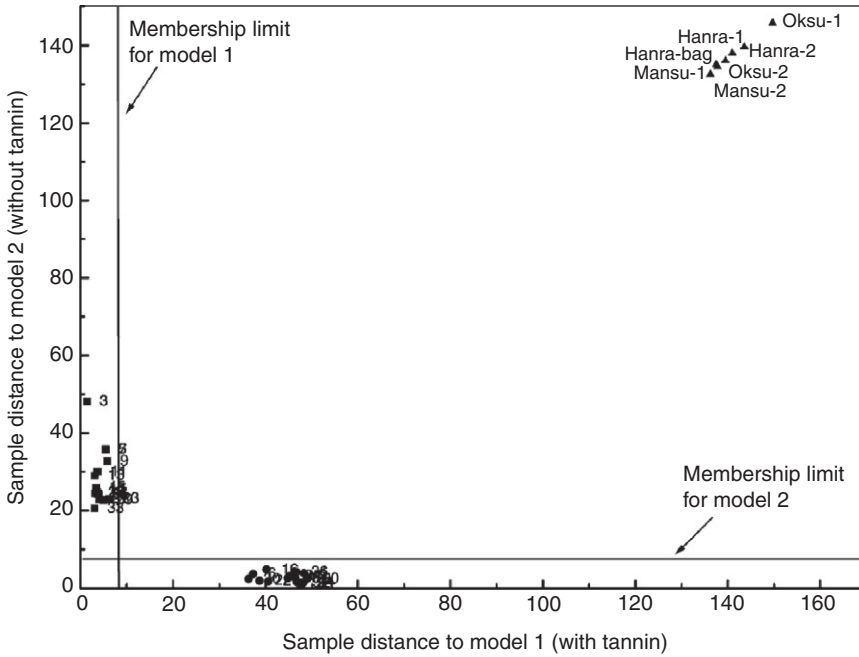

FIGURE 2.23 Coomans' plot of SIMCA models of green teas according to the presence of tannic acid (reproduced from Lvova *et al.*, 2003, with permission).

Ciosek *et al.* (2005) used potentiometric ion-selective sensors for discriminating different brands of mineral waters and apple juices. PCA and ANN classification were used as pattern recognition tools, with a test set validation (Ciosek *et al.*, 2004b). In a subsequent study, the same research group performed the discrimination of five orange juice brands, with the same instrumental device. A variable selection was performed, by means of strategies based on PCA and PLS-DA scores. The validation was correctly performed with an external test set.

Gutes and coworkers presented an automated electronic tongue based on sequential injection analysis (SIA) and linear sweep voltammetry, for the simultaneous determination of glucose and ascorbic acid, by means of ANN regression. The models were evaluated with an external test set (Gutes *et al.*, 2006).

Olsson *et al.* (2006) studied the performances of a mechanically self-polishing electronic tongue based on pulsed voltammetry, for tea analysis. From the PCA scores (see Fig. 2.24), a drift in the measurements is clearly evident. An appropriate row pretreatment of the signals might reduce this effect without loss of useful information.

Beullens and coworkers compared two potentiometric electronic tongues—a commercial instrument and a self-assembled device—assessing

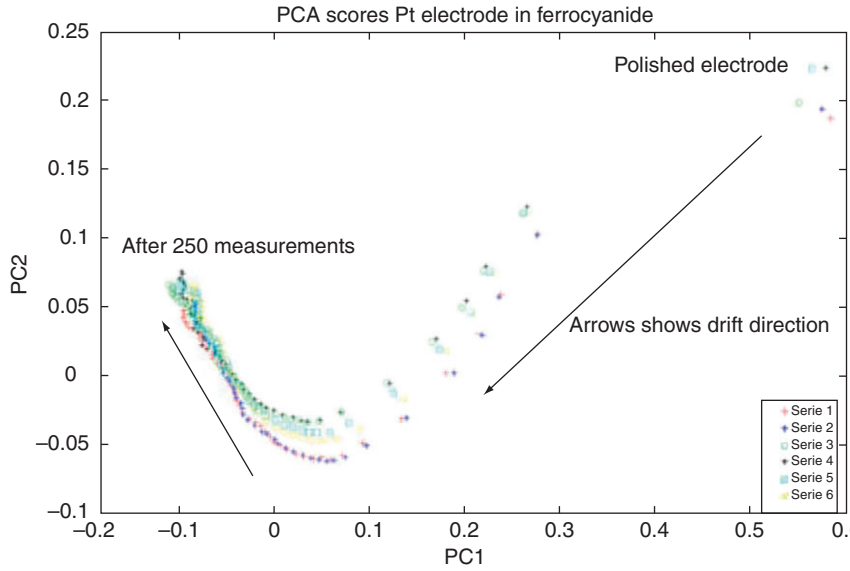

FIGURE 2.24 PCA scores for Pt electrode in ferrocyanide solution. Data represents six series of pulse voltammetry measurements performed with a self polishing device. A reproducible drift pattern is shown (reproduced from Olsson *et al*., 2006, with permission).

their ability in providing information correlated to taste sensations (sweetness, sourness, saltiness, *umami*) evaluated by a panel on tomato samples, and in quantifying simultaneously a number of chemicals: glucose, fructose, citric acid, malic acid, glutamic acid, Na, and K. In addition, the tomatoes analyzed were discriminated according to the cultivar. The pattern recognition techniques applied were PCA, LDA, and PLS. A cross-validation approach was followed for evaluation of the model performances (Beullens *et al*., 2008).

Chen *et al*. (2008) employed a commercial electronic tongue, based on an array of seven sensors, to classify 80 green tea samples on the basis of their taste grade, which is usually assessed by a panel test. PCA was employed as an explorative tool, while *k*-NN and a back propagation artificial neural network (BP-ANN) were used for supervised classification. Both the techniques provide excellent results, achieving 100% prediction ability on a test set composed of 40 samples (one-half of the total number). In cases like this, when a simple technique, such as *k*-NN, is able to supply excellent outcomes, the utilization of a complex technique, like BP-ANN, does not appear justified from a practical point of view.

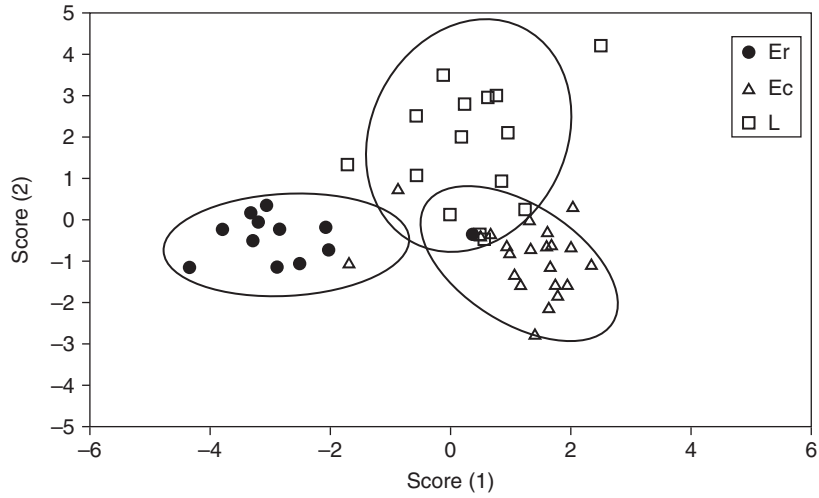

FIGURE 2.25 Score plot on the two LDA discriminant functions, showing the discrimination among honey samples, on the basis of potentiometric data, according to the predominant pollen variety: Er, Erica; Ec, Echium; L, Lavandula (reproduced from Dias *et al.*, 2008, with permission).

Dias and coworkers utilized an array of potentiometric sensors for the classification of honey samples from different Portuguese regions with respect to the predominant pollen type: Erica, Echium, Lavandula. PCA and LDA were employed for the pattern recognition (see Fig. 2.25), after having verified that the variables followed a normal distribution. Cross-validation was applied for evaluating the classification rules, obtaining satisfactory prediction abilities for two classes (about 80%) and poor results for the third one (about 50%) (Dias *et al.*, 2008).

In a further study, Dias *et al.* (2009) studied the deployment of a potentiometric electronic tongue based on an array of 36 sensors, for the recognition of the basic taste sensations and for the detection of fraudulent additions of bovine milk to ovine milk. The signals were processed by means of PCA and LDA (see Fig. 2.26), and the classification rules were evaluated by means of cross-validation. The results presented are excellent for fitting but not very satisfactory for prediction.

Paixão and coworkers described a voltammetric electronic tongue able to distinguish milk treated by means of different pasteurization processes. Furthermore, the method proved to be useful for the detection of hydrogen peroxide additions to milk. Such a fraudulent practice was discovered in 2007 in Brazil. The practice was aimed at reducing the bacterial growth in milk, thus lessening the degradation processes. But, it is

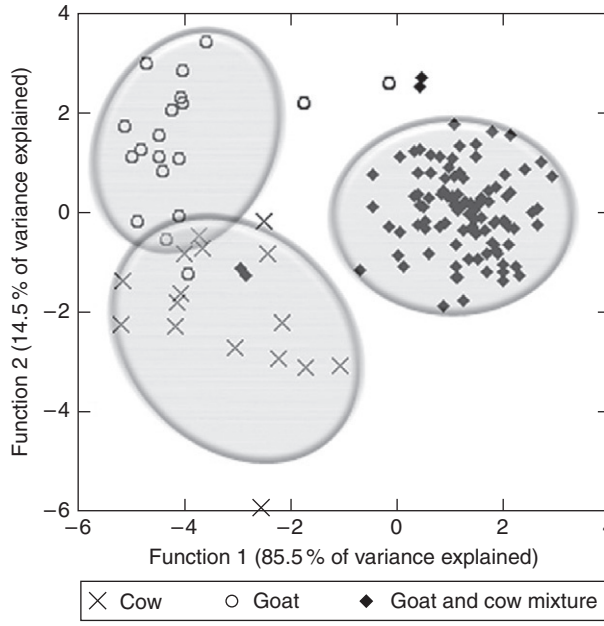

FIGURE 2.26 Score plot on the two LDA discriminant functions, showing the discrimination among skim raw milk samples according to their nature, on the basis of potentiometric data (reproduced from Dias *et al.*, 2009, with permission).

forbidden by law. A simple PCA of the current/potential profiles was able to clearly identify the adulterated milk samples (Paixão and Bertotti, 2009).

Cosio *et al.* (2006) used an electronic tongue system based on flow injection analysis (FIA) with two amperometric detectors, together with the use of an electronic nose, in order to classify olive oil samples on the basis of their geographical origin. Counter-propagation maps were used as classification tools.

Oliveri *et al.* (2009) presented the development of an artificial tongue based on cyclic voltammetry at Pt microdisk electrodes for the classification of olive oils according to their geographical origin: the measurements are made directly in the oil samples, previously mixed with a proper quantity of a RTIL (room temperature ionic liquid). The pattern recognition techniques applied were PCA for data exploration and *k*-NN for classification, validating the results by means of a cross-validation procedure with five cancellation groups.

An interesting issue, in the field of nonspecific analysis, is the fusion of data coming from different analytical techniques. As for artificial tongues, potentiometric and voltammetric data have been employed

jointly (Ivarsson *et al.*, 2001) and also together with conductometric data (Winquist *et al.*, 2000), demonstrating that it is possible to achieve better results than by using the information separately. In other cases, data from electronic tongues and electronic noses have been combined (Winquist *et al.*, 1999), confirming that the two instruments provide complementary information, because data fusion improves the ability of the models.

From a chemometric point of view, the only constraint is that an appropriate column preprocessing, such as autoscaling, is required in order to eliminate systematic differences between variables of a different nature. Then, when the original variables are very numerous, it is possible to join the PCs computed separately for each variable block.

Anyway, using data from different instruments increases the experimental effort, time, and costs of analysis. For this reason, although fusion is always an elegant data-processing procedure, for practical applications the real benefit/cost balance should be carefully evaluated.

VIII. CONCLUSIONS

Artificial tongues represent effective analytical tools able to characterize samples by means of a nonspecific approach. They may provide information useful for many purposes, allowing both qualitative and quantitative applications.

In each case, chemometrics plays an essential role in data processing, which corresponds to the brain elaboration of exteroceptive stimuli in mammalian sensory processes. A clever employment of chemometric tools is thus essential for obtaining reliable and valuable results.

Some basic guidelines can be drawn:

- Evaluate carefully the most appropriate data preprocessing tools (signal corrections, transforms, compression) in order to minimize the amount of unwanted information.
- Always start pattern recognition by performing a preliminary multivariate data exploration: PCA is a perfect tool for this purpose, being useful to visualize structures (groupings, correlation) within the data and to make decisions about the subsequent processing steps.
- Use the simplest possible techniques (possibly linear and with few constraints) for data modeling, instead of applying at all costs the most potent tools available: only resort to more complex methods if the data show complex structures and the basic techniques do not

provide satisfactory results. Anyway, mind that complex methods generally require many constraints to be respected; usually, the first one is the requirement for a considerable number of samples.

- Perform a correct and extensive validation of models, in order to properly evaluate their prediction ability and thus their actual applicability. In particular, mind that if model building involves optimization steps, a three-set validation strategy should be applied.

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