MEDICAL DIAGNOSIS WITH ELECTRONIC NOSES

Di Natale, C.[1,2]; Pennazza, G.[1]; Martinelli, E.[1]; Santonico, M.[1]; Macagnano, A.[2]; Paolesse, R.[2,3]; D'Amico, A.[1,2]

[1] Department of Electronic Engineering, University of Rome "Tor Vergata", Via del Politecnico 1; 00133 Roma; Italy,
Fax: +39 (0)6 2020 519, Email: dinatale@uniroma2.it

[2]CNR-IMM, Via del Fosso del Cavaliere, 00133, Roma; Italy.
[3]Department of Chemical Science and Technology, University of Rome "Tor Vergata", Via della Ricerca Scientifica; 00133 Roma; Italy

Received November 05, 2004. In final form June 1st , 2005

Abstract

Electronic noses are arrays of partially selective chemical sensors. These systems appeared at the end of the eighties as technological attempts to mimic some of the functions of natural olfaction. Although they were introduced as a sort of olfaction model they were soon applied to the qualitative analysis in a number of different fields disciplines such as food analysis, environmental control and industrial processes. Among these fields also medicine was considered, in particular taking into account the diagnostic role that olfaction plays in many traditional medicines. In this paper the use of electronic noses in medicine is discussed with a particular emphasis on the possible use of this technology in telemedicine.

Resumen

Las narices electrónicas son dispositivos que forman parte de ciertos sensores químicos que pueden operan bajo condiciones de relativa selectividad. Estos dispositivos aparecen durante la década de los 80, como un intento de emular las funciones del olfato natural. Estos sistemas fueron rápidamente adaptados para el análisis cualitativo en una serie de procesos relacionados con el análisis de alimentos, control ambiental y procesos industriales. En el campo de la medicina se las empleó como dispositivo de olfato, en el proceso del diagnóstico. En este trabajo se discute el empleo de las narices electrónicas en la llamada " telemedicina".

Introduction

The concept that pathologies may alter the chemical composition of human bodies is at the basis of the clinical chemistry. Up to now, the search for correlation between the concentrations of selected bio-chemical compounds in human liquids (mostly urine and blood) and pathologies driven the research in clinical chemistry and it resulted in many currently available analytical techniques for pathologies diagnosis.

Although most of the activities were concerned with the analysis in corporeal liquids, years ago some researches pointed out that also the atmosphere surrounding the living bodies may contain information about the clinical status of individuals. This was well

known in the past when physicians based most of their diagnosis upon their senses. Among them olfaction played a non negligible role in the process of diagnosis formation.

Analytical studies evidenced the presence of typical compounds in the atmosphere immediately outside the human body and in the breath.

The chemical identity of many volatile constituents of body fluids (such as urine, serum and blood) has been described in the diagnosis of several diseases. Among them diabetes, respiratory viral infections and renal insufficiency deserve to be quoted. In particular, the volatile organic compounds present in expired breath may give a potential source of information about general metabolic conditions and, in particular, on lung physiology. Expired air contains a number of volatile organic constituents which are close to be in equilibrium with a number of compartments within the lung and may arise from endogenous or absorbed volatile substances present in the blood. In addiction, certain substances in lung air may be in equilibrium with alveolar fluid or lining material.

The advent of chemical sensors and the electronic nose technology in the last decade opened the opportunity to reconsider these seminal studies in order to look for some forms of novel diagnostic tools considering the chemical information contained in the volatiles secreted outside the human body.

In particular, electronic noses have been applied in many different fields. Recently, also the medical field was taken into consideration and the application of Electronic Nose to detect diseases has been proposed [1].

Electronic Nose Technology

In the eighties the absence of selectivity, one of the major drawbacks of chemical sensors, was taken into consideration as the basis for a novel instrument able to provide global information about samples. This is somewhat resembling what the human olfaction do with odorants [2]. These instruments are basically arrays of non-selective sensors. Sensors are characterised by a wide spectrum of sensitivity to many odorants, with a large overlap of response towards several compounds. This fundamental characteristic of artificial sensors is similar to that found in natural olfaction receptors [3]. This similarity is the basis on which artificial olfaction systems are developed. The sensors response is not univocally correlated with the concentration of a single compound, but rather, it is a sort of combination of the whole chemical information contained in a sample. The performance of natural olfaction at molecular discrimination and recognition results from a complex sensory signal processing carried out in the olfactory bulb and cortex. In the same way, most of the features of the artificial olfaction are revealed after a proper application of multicomponent data analysis ranging from classical statistics, chemometrics to neural networks [4]. The development of electronic noses has become a well consolidated field of research and several examples of these arrays have been widely reported in the literature. Different instruments are also commercially available on the market [5].

It has to be remarked that in spite of the widely accepted term “electronic nose”, current devices are still far from the structure and functions of natural olfaction senses. The only point in common between artificial and natural system is the strategy of measurement that is largely based on arrays of non-selective sensors. The concept underlying

electronic nose systems has been demonstrated to be independent of the particular sensor mechanism, indeed during the last decade almost all the available sensor technologies have been utilised for electronic noses. Clearly, current sensors are very different from natural receptors. These dissimilarities make the perception of electronic nose very different from that of natural olfaction but, in spite of these, several examples of correlations between sensorial analysis and electronic nose have been demonstrated.

The "LibraNose" Project

The LibraNose Project was started at the University of Rome Tor Vergata in 1994. The first protoype of LibraNose appeared in 1995 as an array of Thickness Shear Mode Resonators (TSMR) also known in literature as Quartz Microbalance sensors. The chemical sensitivity is provided by a molecular film of pyrrolic macrocycles (mostly metalloporphyrins and similar compounds). In the current configuration eight sensors are used [6].

Metalloporphyrins are well known for their important role played in many biological processes. In particular, solid state metalloporphyrins show a large selectivity which can be oriented towards different classes of species by changing the molecular constituents: pyrrolic ring, central metal atom and peripheral substituents [7].

Furthermore, metalloporphyrins, due to their organometallic nature, are particularly eligible for artificial olfaction, indeed most of the odorous compounds are excellent ligands for metal ions. Although very little is known about the structures of the olfactory receptor proteins, it may be argued that many of the receptors are likely to contain metal ions at their active sites [8].

Medical issues for electronic nose applications in medicine

In recent years, the medical field was taken into consideration as a possible application area for Electronic Noses. In this context the attention of some researchers was oriented to the identification of infections in living tissues [9, 10, 11]. On these basis the detection of bacteria related to gynaecological infections [12] and the helicobacter pilory [13] were verified with in-vitro analysis.

Besides the analysis of bacteria cultures growing in controlled media, the analysis of the odour of human body was also attempted. In particular the odour of the body surface and the breath was particularly interesting.

Body odour

Human body odour is the result of the combined action of skin gland, sectating organic compounds, whose regulation is subdied to human hormonal control, and bacterial populations localized at skin surfaces, which live metabolising and transforming organic compounds that they are able to absorb from their external environment. Any variation of this equilibrium is then expected to modify both the nature and the amount of volatile compounds forming the skin odour. It is now widely accepted that some pathologies may result in typical skin odour alteration.

Among these pathologies of great interest is the case of schizophrenia. Several years ago a typical skin odour of individuals affected by schizophrenia was observed [14]. This

feature was related to some physiological modification of internal methabolism resulting in the production of some compounds responsible of the characteristic smell. A fatty acid derivative, the trans-3-methyl-2-hexenoic acid was later reported as the major responsible of this characteristic odour. For this reason this compound was proposed as a marker for the diagnosis of schizophrenia, but this hypothesis was not later confirmed by gas chromatography-mass spectrometry (GC-MS) analysis [15] basically because of the large variability of the concentration among individuals and also due to the fact that an absolute relation between the presence of the compound and the occurrence of the disease was not observed. This original finding did not result in a practical diagnostic tool of schizophrenia.
The application of electronic nose to the analysis of skin odour was investigated [16], these results were the first attempt to start an investigation aimed at detecting the schizophrenia.

Also the application of dogs as "diagnostic tools" was investigated. Recently positive results appeared in the literature concerned with the detection of skin [17] and bladder [18] diseases. These studies show that diseases can be detected from volatile compounds and that a specific training can teach dogs to recognize typical odours such as many other tasks for dogs. Nonetheless, since canine perception is based on more than olfaction it is not completely clear that, in these cases, odour is a sufficient source of information for diagnosis.

Breath odour

In the past years, many studies appeared on breath analysis by combined gas chromatography-mass spectroscopy (GC-MS). They revealed the importance of some classes of volatile in certain diseases. As an example mercaptans and aliphatic acids were found in the breath of patients with cirrhosis of the liver while dimethyl- and trimethylamines were found in the breath of uremic patients.

Breath from lung cancer patients was also examined with the same technique. Evidence of different volatile patterns occurring in diseased individuals has been claimed and the presence of a number of compounds whose presence may be correlated to the lung cancer was found. These compounds are mostly some alkanes (hexane and methylpentane among the others) and benzene derivatives such as o-toluidine and aniline [19]. Although these studies did not result in a diagnostic method due to the complex analysis carried out by GC-MS equipment they demonstrated that the chemistry of breath contains information about the presence of cancer in the lungs.

Also the possibility to detect tuberculosis was studied. The use of electronic nose resulted to the detection of tuberculosis in sputum after an incubation period of 6 hours with an enzyme lipase [20]. Although interesting, this procedure is still not viable for on-line applications, specially for those required by telemedicine applications. Another approach aimed at detecting tuberculosis was followed applying an ion-mobility-spectrometer with an electronic nose oriented data analysis [21]. First results have shown the possibility of a rapid identification of bacterial cultures.

A selected study: lung cancer

In here the results of an investigation aimed at studying the possibility to identify lung cancer affected individuals from the analysis of their breath, is illustrated [22].

A total number of forty-two volunteers, all affected by various forms of lung cancer, have been recruited at the C. Forlanini Hospital in Rome. Thirty-five of them were hospitalised waiting for a surgical treatment. Nine patients have been checked after a surgical removal of a tumour mass from the lung. Two patients were measured before and after surgical operations. Eighteen volunteers have been recruited as reference among the medical and nurse staff of the hospital.

Each subject was required to follow the same diet and the same procedure for mouth hygiene. Measurements have been performed in the morning before any food in-take. Individuals were required to deeply breath in a sterile bag (volume of about 4 L). The samples of breath were then immediately analysed with the electronic nose. All measurements were performed on-site. Each subject was measured twice, and the average measurement was used in the data analysis. In order to minimise the influence of possible instrumental drift, the measurement sequence was randomised. The whole experiment lasted five weeks. Post-surgical patients were checked about one month after the operation, in the occasion of a periodical control at the hospital site.

The data analysis here presented has been performed by using Partial Least Squares-Discriminant Analysis (PLS-DA). Three different classes were considered (lung cancer diseased, reference group, and post-surgery patients).

In this study we were concerned with testing the capability of the electronic nose to correctly classify the groups of subjects. The simplest technique that can be used for the scope is the linear discriminant analysis. The use of Partial Least Squares (PLS) helps avoiding the drawbacks due to sensors correlation. Furthermore, PLS provides a clasification of the sensors data in latent variables that can be plotted to provide a visual representation of the classification properties.

As in any supervised classification techniques, the classes have to be chosen a-priori. The natural choice for the samples in our experiment was to choose three classes including the patients with lung cancer, the healthy subjects, and the patients after the surgery. With this classification scheme a PLS-DA model has been built. The best fitting model included 5 latent variables. Data have been linearly normalised and then autoscaled (zero mean and unitary variance). Figure 1 shows the PLS-DA score plot of the first two latent variables. In the plot about 92% of the total variance of the data is represented. As it can be seen, a clear separation between the data related to patients with lung cancer and the other samples is observed. On the other hand, the samples related to post-surgery and healthy reference show some overlap.

A numerical evaluation of the classification properties can be obtained by considering the cross-validation of the PLS-DA method according to a “leave-one-out” technique. The results indicates that 100% of the lung cancer patients have been correctly classified. 94% of the controls has been correctly classified and 6% of them has been classified as belonging to the post-surgery group. Concerning the samples of the post-surgery group 44% have been classified as belonging to an autonomous class, while 56% as been classified as healthy controls.

It is worth mentioning the data related to the two patients measured twice, before and after surgery. In figure 1 the migration of the data points from the class of lung cancer diseases from the healthy controls is illustrated.

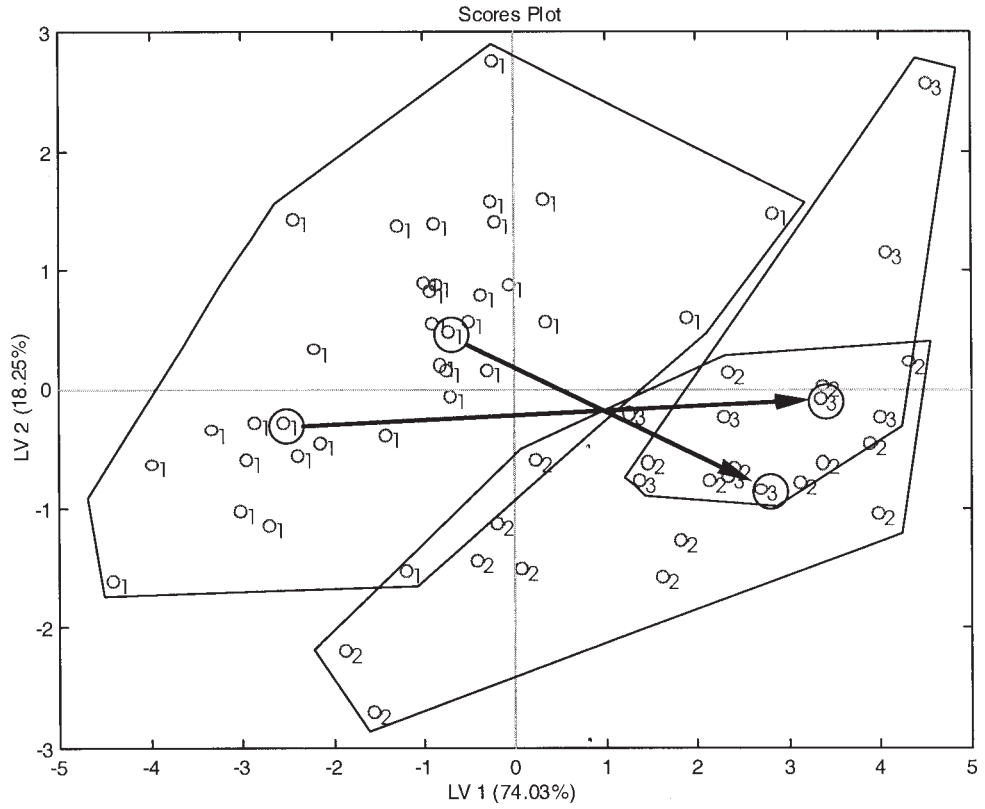

Fig. 1*. PLS-DA score plot of the first two latent variables (74% and 18% of explained variance respectively). Classes are labeled as 1: lung cancer, 2: controls, 3: pust-surgery). Arrows indicate the two patients measured before and after the surgical treatment.*

References

[1] Persaud K.; Pisanelli A.; Evans P., *Medical diagnostics and health monitoring* in *Handbook of Machine Olfaction*, J. Wiley and Sons. Weinheim, Germany, **2003.**

[2] Persaud, K.; Dodds, G., *Nature* **1982**, *299*, 352.

[3] Sicard, G.; Holley, A., *Brain Research*, **1984**, *292*, 283.

[4] Hines, E.L.; Llobet, E.; Gardner, J.W., *IEE Proceedings of Circuits Devices Systems* **1996**, *146*, 297.

[5] www.nose-network.org.

[6] D'Amico, A.; Di Natale, C.; Macagnano, A.; Davide, F.; Mantini, A.; Tarizzo, E.; Paolesse, R.; Boschi, T., *Biosensors and Bioelectronics* **1998**, *13*, 711.

[7] Di Natale, C.; Paolesse, R.; Macagnano, A.; Mantini, A.; Goletti, C.; Tarizzo, E.; D'Amico, A., *Sensors and Actuators B*, **1998**, *52*, 162.

[8] Wang, J.; Luthen-Schulten, A.; Suslick, K.S., *Proc. National Academy of Science* **2003,** *100*, 3035.

[9] Gardner, J.W.; Craven, M.; Dow, C.; Hines, E.L., *Measurement Science and Technology* **1998,** *9*, 120.

[10] Boilot, P.; Hines, E.L.; Gardner, J.W.; Pitt, R.; John, S.; Mitchell, J.; Morgan, D.W., *IEEE Sensors Journal*, **2002,** *2*, 247.

[11] Gibson, T.D.; Prosser, O.; Hulbert, J.N.; Marshall, R. W.; Corcoran, P.; Lowery, P.; Ruck-Keene, E.A.; Heron, S., *Sensors and Actuators B,* **1997,** *44*, 413.
[12] Chendiok, S.; Crawley, B.A.; Oppenheim, B.A.; Chadwick, P.R., Higgins, S.; Persaud, K.C., *Journal Clinical Pathology* **1997**, *50*, 790.
[13] Pavolu, A.K.; Magan, N.; Sharp, D.; Brown, J.; Barr, H.; Turner, A.P.F., *Biosensors and Bioelectronics* **2000**, *15*, 333.
[14] Smith, K.; Thomspon, G.F.; Koster, H.D., *Science*, **1969**, *166*, 398.
[15] Gordon, S.G.; Smith K.; Rabinowitz, J.L.; Vagelos, P.R., *Journal Lipid Research*, **1973**,*14*, 495.
[16] Di Natale, C.; Macagnano, A.; Paolesse, R.; Mantini, A.; D'Amico, A., *Sensors and Actuators B* **2000** *65*, 216.
[17] Pickel, D.; Manucy, G.; Walker, D.; Hall, S.; Walker, *J., Applied Animal Behaviour Science*, **2004**, *89*, 107.
[18] Willis, C.; Church, S.; Guest, C.; Cook, A.; McCarthy, N.; Bransbury, A.; Church, M.; Church J., *British Medical Journal*, **2004**, *329*, 712.
[19] O'Neil, H.J.; Gordon, S.M.; O'Neil, M.H.; Gibbons, R.D.; Szidon, J.P., *Clinical Chemistry* **1988**, *34*, 1613.
[20] Turner, APF; Magash, N., *Nature Review: Microbiology* **2004**, *2*, 161
[21] Bindig, U.; Müller, G.; Katzung, W.; Leonhard, J. Mauch, H., *Proc. of the 9th International Symposium on Olfaction and Electronic Nose*, Rome 29 Spe- 2 Oct, 2002, A. D'Amico C. Di Natale eds., Aracne, Roma, **2002**.
[22] Di Natale, C.; Macagnano, A.; Martinelli, E.; Paolesse, R.; D'Arcangelo, G.; Roscioni, C.; Finazzi-Agrò, A.; D'Amico, A., *Biosensors and Bioelectronics*, **2003**, *18*, 1209.