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Title:
Electronic noses low-ppb calibration procedure in the context of a multicentre medical study

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Abstract

An overview of the scientific literature in the medical field tells us that a large part of electronic nose applications is devoted to breath analysis. A network based study can help testing the validity of this strategy when using many different devices based on identical or different technologies, in view of a use in real clinical practice.

The first step is the instrument calibration using a set of key-compounds. In this work a gas sensor array based on Quartz Micro Balance (QMB) transducers functionalized with metallo-porphyrins (ROTV e-nose), and a Cyranose are used simultaneously in a calibration experiment with three ad hoc selected compounds: ethanol, hexane and ethyl acetate, at concentration levels around 1 ppm. These tests have demonstrated that LODs down to tens of ppb are possible. Moreover, a mapping between the two instruments has been performed through the calculation of a model based on Cyranose data, and applied to the ROTV e-nose data, for the prediction of compound concentrations. This test has shown a good ability in concentrations prediction, with an error lower than 10 ppb.
Introduction

Electronic nose applications in the medical field are nowadays still confined to a research context due to a variety of problems; the most relevant one is the reproducibility of the methodology not adequately tested so far [1-3]. This point is rather critical for breath analysis, which appears to be one of the most promising medical applications of the electronic nose, and the most complex one [4,5]. This is crucial when we take into consideration that breath volatile fingerprint interpretation should be based on reliable and ad-hoc studies, also matching different -omics approaches [6]. In fact clinical practice asks for standard procedures able to give reproducible and equivalent (or at least comparable) results based on different devices used in different places.

This goal can be achieved by means of a careful instrument characterization and comparison to be performed in a dedicated lab, with standard compounds, and for the validation aspects in the hospital, with patients. The calibration procedure has been performed by the authors of this paper in the Philips laboratories (Eindhoven), in the context of U-BioPred (Unbiased BIOmarker in PREDiction of respiratory disease outcomes).

Scientific literature on electronic nose used in medicine is crowded with a large variety of applications, with few examples of basic experiments on the instrument performance in a specific medical field.

The most original approach to the problem of electronic nose characterization was presented by Berna A. et al. [7], who selected the natural olfaction as a ‘gold standard’ for the artificial one, proposing a bio-benchmarking of electronic nose sensors. This approach is fascinating and useful to raise the problem; it shows indeed the gap (as stated by the authors) between natural and artificial olfaction, but what we need for the specific field of application is to state the real performance, in spite of its limitation with respect to nature. Looking at the specific field of breath analysis a useful contribution can be found in a work which shows the limit of detection of a carbon-black polymer based gas sensor array with respect to selected breath biomarkers [8]. The result obtained (80 ppb – 240 ppm) is a good reference for the present work and it is discussed in the conclusions. Besides, other papers can be found on e-noses comparison: one on mapping between data-sets [9], and one on merging [10] all the data obtained with different electronic noses.
Two different electronic noses have been used: Cyranose [11] and the Torvergata electronic nose (ROTV ENOSE) [12]. Sensor performance has been experimentally determined with respect to the selected compounds with respect to ROTV ENOSE and a mapping has been carried out between the data collected with the ROTV ENOSE and the Cyranose.

An important point was related to the definition of a standard calibration methodology to be adopted for a system based on a given gas sensor array.
Experimental

**Sensor arrays**

Tor Vergata e-nose and Cyranose 320 have been used as gas sensor arrays. The last version of the electronic nose designed and fabricated by the University of Rome “Tor Vergata” (ROTV ENOSE) can accommodate up to eight quartz microbalance (QMB) sensors [13]. They were functionalized with molecular films of different metalloporphyrins synthesized by the Dept of Chemical Science and Technology of the University of Rome “Tor Vergata” [14]. The metalloporphyrins used as chemically interactive materials (CIMs) by ROTV ENOSE for these experiments are the following: Cu-Butioloxy-Tetra Phenyl Porphyrin (Buti-TPP), Co-Buti-TPP, Zn-Buti-TPP, Mn-Buti-TPP, Fe-Buti-TPP, Sn-Buti-TPP, Ru-Buti-TPP, Cr-Buti-TPP. The flexibility of the system permits to convey the sample directly into the chamber bypassing the pneumatic apparatus. This set-up was used [15] in order to avoid interferences with other devices (pump, valve, proportional electro-valve) thus allowing the system to work in the best experimental conditions for calibration purposes.

Cyranose 320 is a portable electronic nose based on 32 polymer carbon black composites, manufactured and commercialized by Smith Detection (USA, Pasadena). For further specifications see the user manual.

**Calibration system setup**

In order to perform measurements in the concentration range below 1 ppm, a strategy based on permeation tubes (Fine Permeation Tubes, ME, Italy) has been utilized. This setup was already used with ROTV e-nose [15]. Here the formulas used for the gas concentration calculus as proposed by permeation tube data sheet [16] is reported: 

\[ C(\text{ppm}) = C(\text{ng/sccm}) \times K \]

where \( C \) is the concentration value, \( K = (24.46)/\text{molecular weight} \), and 24.46 is the molar volume in litres @ 25°C, 760mmHg.

Figure 1 shows a snapshot of ROTV ENOSE responses. The sensors are constantly exposed to a reference air (Nitrogen, blue line in the figure for cleaning phase) and the successive exposure to the sample (red line) results in a negative shift of the resonant frequency (positive shift of the conductance values for the Cyranose). The shifts are considered as the response features for each sensor. All the registered frequency variations (conductance variations for the Cyranose) make up the two e-
noses datasets elaborated to obtain the results presented in the following.

Volatile compounds selection
The compounds were selected based on safety profile and on physical-chemical properties in order to cover a range of hydrophilia and polarity at proper azeothrope boiling point. We selected ethanol (hydrophilic, polar), hexane (hydrophobic, apolar) and ethyl acetate (intermediate hydrophilic and polar properties).
Results and Discussion

E-Nose sensors performances
A series of parameters has been calculated for each measured compound; these parameters have been used to assess the performances of the sensors.
Cyraonose characteristics are granted by Smith Detection and reported in literature: the handbook of machine olfaction [17] accounts for a Limit of Detection (LOD) of 0.1% of the standard vapour pressure, which means an LOD of 0.1-10 ppm (depending on the compound), as confirmed by one of the already mentioned papers [8] testing carbon black polymer sensors (the same used by Cyranose).
Before mapping between the two system data, ROTV e-nose has been tested with low concentration levels (round and below 1 ppm).
For each of the eight sensors the following characteristic aspects have been considered: response curve (QMB frequency shift versus VOC concentration), sensitivity (slope of the linearized response curve), experimental Limit of Detection (LOD) determined around the left end part of the response curve, theoretical LOD (estimated considering a signal to noise ratio S/N equal to 1), the predictive power of the sensor array.
Ethanol
The used concentration levels were: 1.3ppm, 0.975ppm, 0.67ppm, 0.325ppm, 0.163ppm, 0.065 ppm. The response curves, one for each sensor, are reported as example in the figure 2.
The error bar (reported for each point) evidences a good reproducibility; thus, the relative standard deviation does not affect the discrimination among the measured concentration levels. In fact the standard deviation results smaller than the difference calculated between contiguous concentration levels (among the six measured in this calibration test).
Response curves are not linear and they can be fitted prima facie by a Langmuir Isotherm curve.
Each of the eight sensors is sensitive to ethanol, with eight different sensitivity values. This confirms the peculiarity of the sensor array, designed to be not selective and to show different sensitivities.
Sensitivity goes from a minimum value of about 20 Hz/ppm to a maximum value of about 110 Hz/ppm. This substantial reduction of the sensitivity shows the non-linear
behavior of the sensor response, which seems to enter a saturation-like region with concentration level increasing up to 1 ppm.

The LOD experimentally measured is of 0.065 ppm, with an S/N ratio, still larger than 1. In fact, this is the lowest measured level of ethanol concentration, and the relative response values range from a minimum of 6 to a maximum of 12 Hertz.

Given the noise level of the sensor response of about 3 Hz, the theoretical LOD is 0.0325 ppm.

The Predictive power of the sensor array has been evaluated by calculating the Partial Least Square Discriminant Analysis (PLS-DA) model, using the leave one out criterion as cross-validation technique. Figure 3 shows the good performance of the system: an almost total correlation between real and predicted concentration values was observed. The Root Mean Square Error Calculated in Validation (RMSECV) is 0.077 ppm.

**Hexane**

The measured concentration levels were: 1.13 ppm, 0.847 ppm, 0.565 ppm, 0.282 ppm, 0.141 ppm. Response curves (here not shown) are not linear, but, prima facie, they can be considered as linear. The good reproducibility observed for ethanol is here confirmed for hexane. The sensitivity values of each sensor with respect to hexane, have been calculated according to the linear approximation of the response curve: sensitivity ranges from a minimum value of about 18 Hz/ppm to a maximum value of about 32 Hz/ppm for the different eight sensors.

The LOD experimentally measured is 0.141 ppm. In fact, this is the lowest measured level of hexane concentration, and the relative response values range from a minimum of 10 to a maximum of 19 Hertz. Given the noise level of the sensor response of 3 Hz, the theoretical LOD is 0.0423 ppm.

The Predictive power of the sensor array has been evaluated by calculating a Partial Least Square Discriminant Analysis (PLS-DA) model, using the leave one out criterion as cross-validation technique: an almost total correlation between real and predicted concentration values was observed. The Root Mean Square Error Calculated in Validation (RMSECV) is 0.054 ppm.
**Ethyl Acetate**

The measured concentration levels are: 1.37ppm, 1.03ppm, 0.685ppm, 0.342ppm, 0.171ppm. The good reproducibility already observed for the other two compounds is here confirmed for ethyl acetate. Response curves (here not shown), prima facie, can be considered as linear. The sensitivity value of each sensor with respect to ethyl acetate, have been calculated according to the linear approximation of the response curves. Sensitivity ranges from a minimum value of about 24 Hz/ppm to a maximum value of about 47 Hz/ppm.

The LOD experimentally measured is 0.171 ppm. In fact, this is the lowest measured level of ethyl acetate concentration, and the relative response values range from a minimum of 5 to a maximum of 13 Hertz. Given the noise level of the sensor response of 3 Hz, the theoretical LOD is 0.103 ppm.

The Predictive power of the sensor array has been evaluated by calculating a Partial Least Square Discriminant Analysis (PLS-DA) model, using the leave one out criterion as cross-validation technique: an almost total correlation between real and predicted concentration values was observed. The Root Mean Square Error Calculated in Validation (RMSECV) is 0.025 ppm.

Similar data, here not included, have been collected by Cyranose, whose overall performance can be also found in literature [8].

**Data fusion and mapping between the two e-noses**

The data collected with the two instruments have also been analyzed together. To this purpose two ways can be followed: data fusion and mapping.

With the data fusion approach all the data have been used together in order to build a model able to predict compounds concentrations. Table 1 summarizes the results obtained: The Root Mean Square Error in Cross Validation (RMSECV) calculated for the ROTV ENOSE data is smaller than one order of magnitude compared to the one calculated for the Cyranose for all the three compounds. Besides, the fusion of the two dataset allows to build a model which is more performant for ethanol concentration prediction. This last result suggests that the comparison between the two e-noses cannot be based only on these three compounds, because the sensitivities and selectivities of the different sensors could be hopefully complementary rather than antithetic, being not selective in principle.
A good way to test this option consists of finding out whether or not the Cyranose data can be used as training set for the test dataset collected with ROTV ENOSE. Results are surprisingly good. Figures 4, 5, and 6 report an almost total correlation between real and predicted concentration values for ethanol, ethyl acetate and hexane respectively. It is worth remarking that, in spite of a good correlation between real and predicted value, error gets worse of one order of magnitude with respect to the LOD calculated for each single instrument. This result is important to state that, even if the feasibility of a mapping between e-noses confirms that a volatile fingerprint is a common and reproducible information source also when using different devices, the best performance can be obtained by each instrument when used alone.
Conclusions

The results obtained represent a rather useful basis to complete a sort of datasheet to be associated to the electronic noses system. This is mandatory for each instrument, but surprisingly this duty has been neglected for the electronic nose in favor of a non-selective and ‘olfactive’ approach to VOCs analysis. This is justified by the large variety of applications where e-noses have been applied, and by a lack of information about characteristic compounds to be tested in term of sensitivity, resolution and limit of detection.

Here ROTV ENOSE and Cyranose have been shown to be able to detect concentration levels far below 1 ppm, which seems to be low enough for respiratory diseases studies; this result is comparable to and lower than the ones already cited [8]. Certain VOCs identified for specific diseases have been quantified to be meaningful down to ppt, so that preconcentration techniques could be applied to breath sampling apparatus to support e-nose classification abilities.

Moreover, ROTV e-nose and Cyranose 320 can be used together in a multicenter medical study, because a mapping strategy can be successfully applied between the two datasets. In view of a clinical application mapping between eNoses will be essential, in order to exchange training and validation data between centers e.g. by internet platforms. Even though proposals for mapping between eNoses have been made [9], such procedures still need to be validated in the experimental setting.

Overall, the present study represents a preliminary step towards quality assurance in clinical research and future application of eNoses in the medical setting.
Conflict of interest statement

We hereby declare that no conflict of interest related to the publication of this manuscript exists.

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References


[16] www.finepermeation.it

Tables captions

Table 1
This table reports the performances of ROTV e-nose (here addressed as TEN) and of Cyranose in terms of Root Mean Square Error in Calibration and in Validation expressed by the PLS-DA model calculated on the two data sets. The last two column are referred to the model built on the data fusion of the two sets.

Figure captions

Figure 1
Dynamic response curves obtained by the ROTV e-nose sensors when exposed to an increasing (0.67, 0.975 and 1.3 ppm) and then decreasing (0.67, 0.975 and 1.3 ppm) concentration of ethanol respectively.

Figure 2
Calibration curves of each ROTV e-nose sensor for the concentration levels tested for ethanol: 1.3ppm, 0.975ppm, 0.67ppm, 0.325ppm, 0.163ppm, 0.065 ppm. reports the curves relative to the eight sensors (Cu-Buti-TPP, Zn-Buti-TPP, Fe-Buti-TPP, Ru-Buti-TPP, Co-Buti-TPP, Mn-Buti-TPP, Sn-Buti-TPP, Cr-Buti-TPP).

Figure 3
Plot of the measured ethanol concentration values vs the ethanol concentration values predicted by a PLS-DA model calculated on the ROTV e-nose data.

Figure 4
Plot of the 12 measurements of ethanol concentration performed with the two e-noses. The concentration value of each measurement is reported on the y-axis. The blu * are the real concentration values while the red + are the concentration values predicted by a PLS-DA model trained on the Cyranose data and tested on the ROTV e-nose data.

Figure 5
Plot of the 10 measurements of ethyl acetate concentration performed with the two e-noses. The concentration value of each measurement is reported on the y-axis. The blu * are the real concentration values while the red + are the concentration values predicted by a PLS-DA model trained on the Cyranose data and tested on the ROTV e-nose data.

Figure 6
Plot of the 11 measurements of hexane concentration performed with the two e-noses. The concentration value of each measurement is reported on the y-axis. The blu * are the real concentration values while the red + are the concentration values predicted by a PLS-DA model trained on the Cyranose data and tested on the ROTV e-nose data.
<table>
<thead>
<tr>
<th></th>
<th>ROTV ENOSE RMSEC</th>
<th>ROTV ENOSE RMSECV</th>
<th>CYRANOSE RMSEC</th>
<th>CYRANOSE RMSECV</th>
<th>CYRANOSE + RMSEC</th>
<th>CYRANOSE + RMSECV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (ppm)</td>
<td>0.034</td>
<td>0.079</td>
<td>0.093</td>
<td>0.106</td>
<td>0.007</td>
<td>0.052</td>
</tr>
<tr>
<td>Ethyl acetate (ppm)</td>
<td>0.005</td>
<td>0.024</td>
<td>0.027</td>
<td>0.26</td>
<td>0.015</td>
<td>0.062</td>
</tr>
<tr>
<td>Hexane (ppm)</td>
<td>0.032</td>
<td>0.054</td>
<td>0.225</td>
<td>0.249</td>
<td>0.027</td>
<td>0.119</td>
</tr>
</tbody>
</table>
Figure 1: Graph showing Delta f (Hz) over Samples.

The graph displays multiple curves labeled QMB1 to QMB8. The x-axis represents samples, and the y-axis represents Delta f (Hz).
Figure 4

Ethanol concentration prediction

- Y-axis: Ethanol concentration [ppm]
- X-axis: Measurements

The graph shows a scatter plot of ethanol concentration predictions against measurement numbers.