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Thermal Desorption System for Breath Samples Analysis from Colombian Patients with Gastric Cancer

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This work describes the development of a low cost thermal desorption system to analyze a set of 31 exhaled breath samples previously acquired from CA and control patients (i.e. with gastritis and ulcer), which were concentrated using Tenax tubes in order to remove the moisture and trap the volatile compounds. The samples were stored at a temperature of 4°C for further analysis. The proposed system allowed that the volatile compounds were trapped inside the tubes to be extracted and sent to a measuring chamber with a gas sensor array sensitive to these compounds. The overall detection system composed of the measuring chamber, a high-precision power supply, advanced high-resolution data acquisition equipment and a computer that acquired and supervised the sensor responses. Once the information was acquired, different pre-processing (normalization) and data processing techniques such as: Principal Component Analysis (PCA), Probabilistic Neural Network (PNN) and Support Vector Machine (SVM), were applied for the analysis and data classification of the exhaled breath. The thermal desorption system was able to extract the volatile compounds emitted from the breath, reducing the humidity of the samples to increase the selectivity, sensitivity and the performance of the system. A 99,44 % of the total variance by using PCA analysis was achieved and a 93.54 % of classification success rate using SVM was obtained.

1. Introduction

Actually, different studies have been developed through methods for the diseases detection by breath analysis, specially for respiratory diseases as lung cancer (McWilliams et al., 2015), asthma (Kaushal and Mudhalwadkar., 2015), COPD (Zubaydi et al., 2017) as others, where meaningful differences are found on comparing the Volatile Organic Composites (VOC's) of sick persons and healthy persons. At the same time, making comparisons with different levels of the disease (i.e., critical patients, moderated patients). Studies have also been done with other kind of diseases, such as prostate, colon-rectal, breast and esophagus cancer, multiple sclerosis as others, using simple nanosensors array. In other hand, interesting results have been obtained not only to differentiate between healthy and cancer patients, but to distinguish between breathe patterns of different kinds of cancer with the same statistic analysis. Some studies have been done by using systems based on a group of sensors (TMNA-NOSE), that are composed of metallic nanoparticles and/or carbon nanotubes functionalized with organic molecules and hydrophobic properties (it means, to reduce the high water content in the breathe), in order to detect and analyze small concentrations of organic volatile particles that are normally emitted on breathing. These concentrations are around one million part, since the nanosensors experience changes when they are exposed to the VOC's; in this case, the alveolar air it is translated into different patterns. These VOC's patterns can be used as non invasive biomarkers of several biochemical processes that are presented in diseases (Peng et al., 2010). In last years, these studies have been focused to explore the use of the exhale breathe on the early detection of gastric diseases, (Amal et al., 2013), particularly, gastric cancer in different states (Xu et al., 2013), present results that allow to distinguish between gastric cancer and benign gastric conditions, using sensors based on nanomaterials, taking as reference samples of 130 patients who came from hospitals of China and Latvia, with different clinical conditions (i.e., cancer, ulcer, gastritis, among other less severe critical conditions) in this work predictor models such as: Discriminant Factor Analysis (DFA) were also used to build the classifying models.

Taking as reference the last study, present proposal was done with a group of Colombian patients wit gastric cancer and under control (persons with similar diseases), using an electronic nose system, based on gold nanoparticles sensors (GNP), obtaining promissory results. In order to increase sensibility, selectivity and repeatability of the sensors response, it was suggested the use of pre concentration methods and the development of a thermal desorption system to adapt it to the multisensorial system.

2. Thermal Desorption Methods

Considering some limits for sensibility and selectivity of the Electronic Smell Systems (ESS), some strategies were described in this study to improve these two aspects. In this case, pre-concentration method and the thermal desorption system were outstanding, since they were fundamental parts to realize the proposed design.

2.1 Preconcentration

Sensibility of the device can be increased by a pre-concentration stage. With this kind of methods, it is possible to couple a measuring device (e.g. ESS) and also increase the selectivity, since when interesting compounds are pre-concentrated, these compunds are filtered on indirect way. In this study, an absorption tube ORBO™-420 Tenax, manufactured by Sigma-Aldrich (Supelco), 2015 was used. This absorbent are widely used for the absorption of VOCs in the air and can also be desorbed at temperatures between 200-500 ºC. Tubes have two zones of A/B polymer material (two beds of the same absorbent) selective and separated by fiberglass or foam, for sampling of gas or steam. The construction with double cover permits to catch any sample in the smallest zone (B) (See Figure 1)

Figure 1: Absorption ORBOTM-420 Tenax.

2.2 Thermal Desorption

Thermal desorption is used for VOCs analysis on gas samples. Samples on gas phase are collected in tubes with specific materials to trap differents compunds in order to be analyzed, then desorption tubes are introduced within a thermal desorption device that heat the tubes to release the VOCs previouly trapped. Afterwards, compounds are transfered by an inert gas (e.g, Nitrogen) towards the detection instrument.
In this study, a low cost and portable desorption system was developed to be coupled directly to the multi sensorial system.

3. Materials and methods

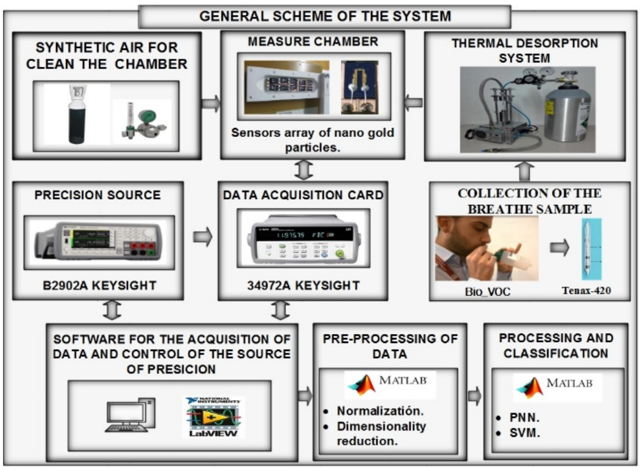

Figure 2: Electronic Smell System scheme.

Figure 2 shows the work sequence and the main modules that compose the ESS that was used in the study with Colombian patients. The system includes a sensors matrix of gold nanoparticles (AuNPs) functionalized with organic ligands, a high precision power supply, data acquisition card and purge system for the sensor chamber and a computing system that allows to apply data processing (pattern recognition methods), to classify and quantify whatever volatile compounds. For data acquisition and processing an application using Labview software coupled with Matlab were used to obtain the results.

3.1 Sampling System (Collection of the breathe sample)

Exhaled breath samples were collected at the Hospital Universitario de la Samaritana-Bogotá (GASTROSUR SAS) in the same clinical environment under control, by using the Bio-VOC device, and they were pre concentrated into adsorption tubes called ORBO™-420 Tenax. Tubes were stored at 4°C temperature, for further analysis. A total of 31 breath samples were collected. 15 of them correspond to patients diagnosed with gastric cancer (CA) and 16 control patients (C) with gastritis, ulcers and other gastric conditions.

3.2 Thermal Desorption System

Figure 3a illustrates the devices that compose the Desorption System. Tenax tube is set into the heating capsule. Then, with an electric resistance it was heated until it reaches the desorption temperature around 200-250ºC, which corresponds to the accurate range for the extraction of volatiles. A temperature sensor was placed into the capsule to keep and control the temperature using a Proportional Integral Derivative (PID) controller. Figure 3b illustrates the control system response wich is characterized with a temperature of 250 °C.

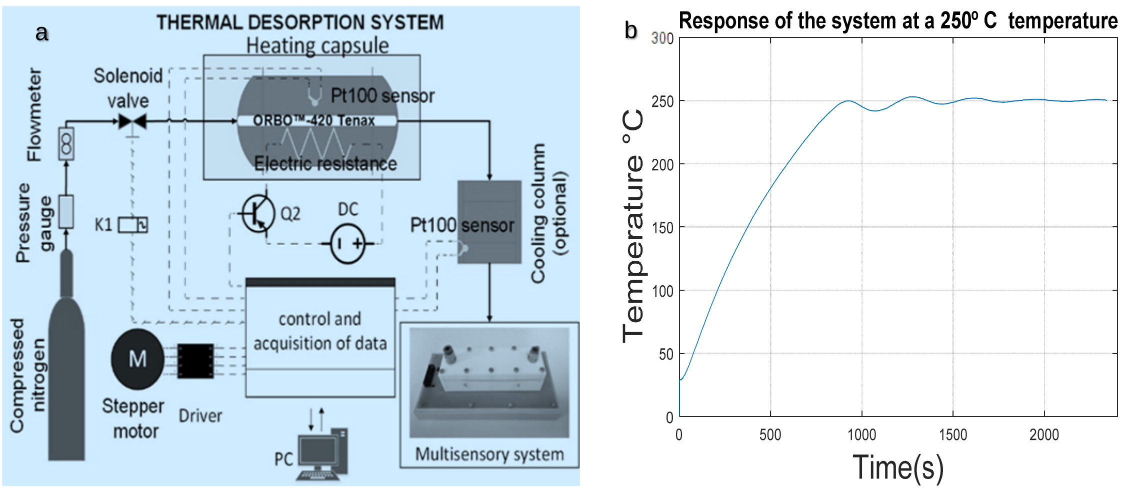

Figure 3: a) Thermal Desorption System. b) Control system response at 250 ºC.

After reaching desorption temperature, it kept itself for an estimated time to release the VOCs contained by the adsorbent tubes, which were extracted from the tube when an electric valve was open, allowing so the pass of pure Nitrogen to the input of the heating capsule with the tenax tube, to transfered the VOCs to the measuring chamber. During this pressure stage (0-50 psi) and the flow (50-500 mL/min), they were controlled with the manometer and the flow meter. A control card was placed at the desorption system for control of all components and making the communication with a graphical user interface, to supervise the temperature of the capsule; times of each one of the stages were fixed by the application.

Figure 4a, shows the functional prototype of thermal desorption system, which was made with stainless steel SAE-316, SAE-316L in order to avoid pollution, oxidation and any damage of the components. Electronic components were located inside (Bottom) the system.

Figure 4b, depicts the transverse section of the capsule, where the body and the cover of the capsule were developed with an insulating packaging resistant to high temperatures, avoiding that the sample may be polluted by external agents or could be diluted during the desorption.

The insulating packaging helps the Nitrogen flow to circulate inside the tube so that VOCs to be transfered.

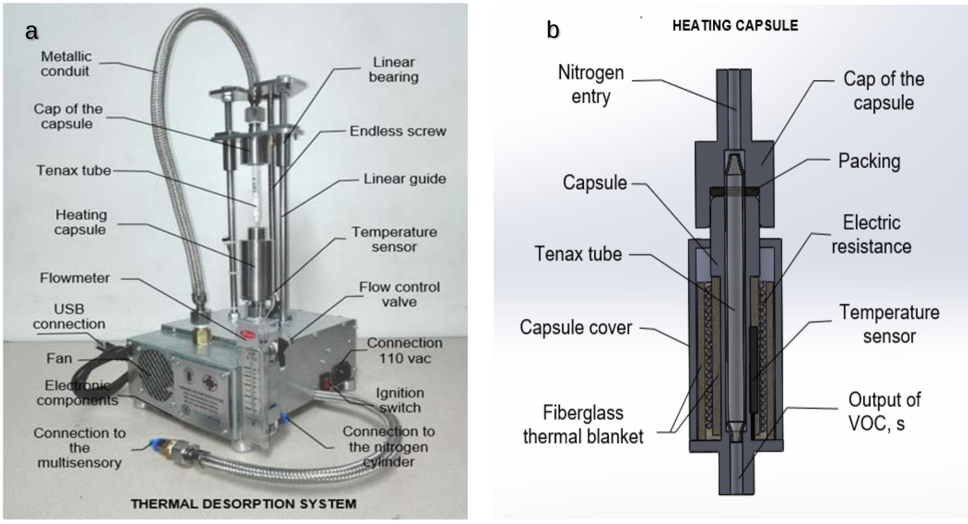

Figure 4: a) Thermal Desorption System. b) View of the transversal section of the de la capsule.

3.3 Measure Chamber.

Figure 5 shows the measure chamber, which has a arrayof 10 Gold Nano-Particles gas sensors (i.e, Resistive type) functionalized with different organic ligands (See Figure 5). GNPs can generate the electric conduction, while the organic material reacts with the breath components, which makes to vary the internal resistance on function of the volatile components (Gualdrón et al., 2017).

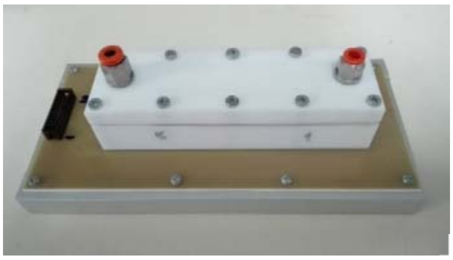

Figure 5: Measure Chamber

3.4 . Patterns Recognitio

The sensor parameter that was used in the data processing was the electric current, Also, the mean value was extracted of each sensor response (from 6 cycles) in order to reduce and obtain the relevant information of data set. Once data were obtained in the acquisition stage, different technics for data preprocessing (i.e., normalization and dimensionality reduction) (Moreno et al., 2009) and data processing (PCA) were applied. The last one is a common pattern recognition method used in multivaried analysis and a lineal-no supervised and supervised algorithm (Sánchez, L., Osorio, G., 2008). The classification methods specifically the PNN (Monteiro et al., 2018) and SVM classifier, were implemented by means of cross validation technique "leave one out", in terms of the number of True Positives (TP), True Negatives (TN), False Positive predictions (FP) and False Negatives (FN). The accuracy, sensitivity and specificity were finally calculated Eq(1). The classifier models were obtained using the Matlab platform.

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \qquad Sensitivity = \frac{TP}{TP+FN} \qquad Specificity = \frac{TN}{TN+FP} \qquad (1)$$

4. Results and Analysis

4.1 . Electric Response of the Sensors

Figures 6a and 6b show the responses of Sensors 5 and 9, which presented meaningful changes on the electric response, due to VOCs could be identified by means of the generated signals of each sample (cancer and control). At the end, they showed a different behavior of sensor response. For analysing the electric response of the sensors, the desorbed VOCs were detected with the measure chamber. The main reason to show only two sensors was due that these sensors were more selective and stable in comparison with the rest. However, the sensitivity, behaviour and perfomance of each one of them were quite similar.

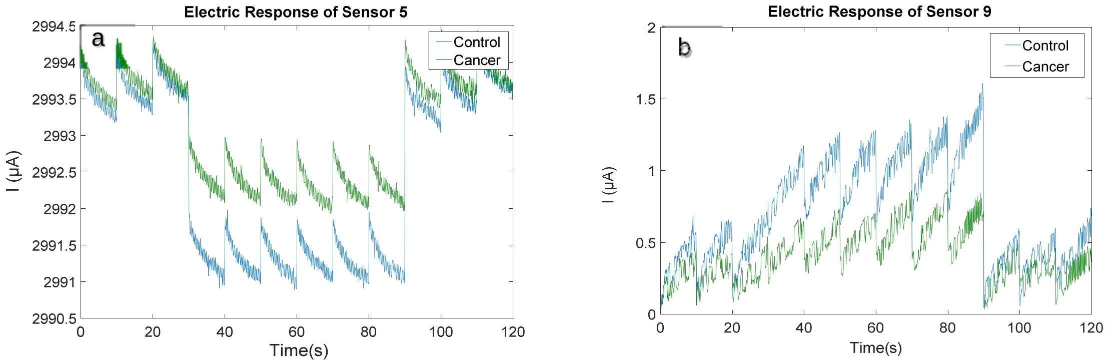

Figure 6: *a) Electric Response of Sensor 5. b) Electric Response of Sensor 9.*

4.2 . Non supervised analysis (PCA)

The PCA analysis in non-supervised mode was applied to the normalized data, where an excellent performance was achieved. Figure 7 shows the discrimination between two categories that correspond to each class, a 99.44% variance was obtained using two first PCs. On X Axis the total viance from the data set was reached.

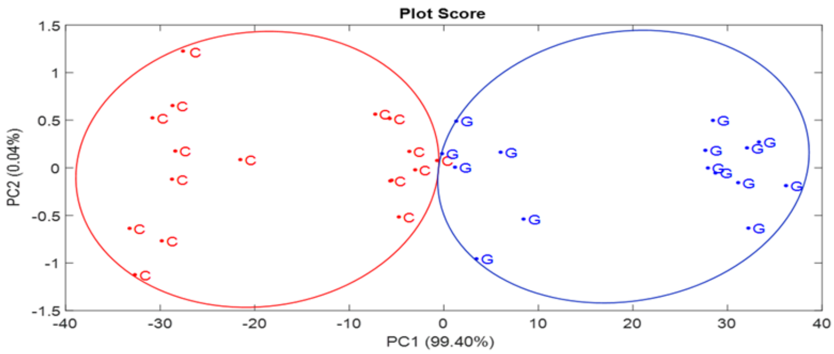

Figure 7: *Analysis of principal components (PCA).*

4.3 . Supervised Analysis (Methods for Patterns Recognition)

In this case, an accuracy percentage was calculated, reaching a 93.54% succes rate of classification with the PNN as well as SVM, using "leave me out". Two samples were missclassified by two models, in the border between two observed clusters (See PCA plot). Table 1 presents obtained results with classifiers methods.

Table 1: Results with classifiers models

Statistic Parameters	Crossed Validation (Leave one out)	
	SVM	PNN
True positives	14	14
True negatives	15	15
False positives	1	1
False negatives	1	1
Accuracy (%)	93.54	93.54
Sensitivity (%)	93.33	93.33
Specificity (%)	93.75	93.75

5. Conclusions

Bio–VOC Sampler and absorption tubes ORBOTM-420 Tenax, demonstrated being suitable for conditioning of breath samples, leading a excellent results with thermal desorption system coupled to ESS.
PCA analysis and classifier models (PNN and SVM) showed an excellent response on discrimination between two categories of the study (cancer and control patients). With the classifiers models applying “leave one out” cross validation technique a 93.54% of accuracy, 93.33% of sensitivity and 93.75% of specificity were obtained.
The automatic process of thermal desorption system, besides of making sure the same conditions for all measures, avoids the sample to be polluted by external agents or to be diluted during desorption, where the little intervention of the analyst, minimizes the pollution risk and lose of analytes, contributing to the repeatability on the sensors response. Besides, it makes longer the sensors life because they are not exposed to the humidity. The percentage of the defined pattern for the assessment by endoscopy was near to 95%, if it is compared with the obtained ones with the evaluated models (93.54%), where it can be conclude that results with the developed system for detection of gastric cancer using the multi sensorial system are promising and can certainly become a parallel strategy for the technics actually used.

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