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Wize Sniffer 1.0: development of a new portable device designed for selective olfaction

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Introduction

Human breath is largely composed of oxygen, carbon dioxide, water vapor, nitric oxide, and numerous volatile organic compounds (VOCs). The type and number of the VOCs in the breath of any particular individual will vary but there is nonetheless a comparatively small common core of breath which is present in all humans.

To date, more than 1000 molecules and different compounds have been identified to be present in exhaled human breath. Among these:

- molecules or their metabolites originating from inhaled air, from dermal absorption, from foods and beverages (exogenous molecules);
- molecules produced by anabolic or catabolic reactions that occur in tissues or cells throughout the body. These molecules, that are endogenous, are present in breath relative to their types, concentrations, volatilities, lipid solubility, and rates of diffusion as they circulate in the blood and cross the alveolar membrane.

Approximately, 35 of the identified compounds in the exhaled breath have been established as biomarkers for particular diseases and metabolic disorders. It means, changes in the concentration of the molecules in these VOCs could suggest various diseases or, at least, changes in the metabolism.

Such biomarkers have been identified using instrumentations such as gas chromatography (GC) or electronic nose (e-nose). GC is very accurate but is expensive and not portable, its sampling and assaying processes are complicated and time consuming (about one hour for one sample), and its results require expert interpretation. E-nose is cheaper and faster (requiring only 30 minutes for one sample) but it is often used outside of medicine, in fields related to food, chemistry, fragrances, security, and environment. Recently, e-nose has gradually been used in medicine for the diagnosis of renal disease, diabetes, lung cancer, and asthma, but it can identify only one particularv disease. One reason for the limited applications of e-noses in breath analysis might be the design of commercial e-noses for broad applications rather than for breath analysis specifically.

As a consequence, in recent years, the necessity to develop a portable device for breath analysis, easy to use, and feasible for patients living far from medical structures or physicians.

In this work, we describe the design and functionality of the first prototype of a new portable device for breath analysis limited to an effective number of substances, the so called **Wize Sniffer 1.0**. In particular, it will be described:

- the design of the hardware platform, including the Arduino Ethernet board, the circuit for the connection between Arduino board and an array of commercial gas sensors;
- the implementation of the communication protocol between sensors and the notebook (laptop);
- the functionality of the Wize Sniffer device with all the gas tests;
- data analysis.

The idea of the Wize Sniffer takes place within **SEMEOTICONS** (**SEMEiotic Oriented Technology for Individual's CardiOmetabolic risk self-assessmeNt and Self-monitoring**, www.semeoticons.eu), an European triennial project started on November 2013 whose central idea is to exploit the face as a major indicator of individual's well-being and to assess a number of computational descriptors to atherosclerotic cardiovascular diseases that are leading causes of mortality worldwide. These descriptors can be expressed involving (i) morphometric, biometrics and colorimetric of the face; (ii) spectroscopic analysis of skin and iris, of sub-cutaneous substances and the function of subcutaneous tissues, and (iii) compositional analysis of exhaled breath.

Such analysis will be made through an innovative multisensory system integrated into a hardware platform having the exterior aspect of a mirror: the so-called "Wize Mirror".

Within the SEMEOTICON Project by the Wize Sniffer, we intend a hardware/software tool for the analysis of volatile organic compounds of breath, to be integrated in the Wize Mirror. The Wize Sniffer should be able to provide useful information about the **"breathprint"**, the analog of fingerprint for the **state of health** of an individual, and to give, in case, feedbacks about alcohol intake and smoking habit. For this purpose, as described also in [2], the Wize Sniffer 1.0. should be able to detect **carbon monoxide** (the major component present in tobacco fumes), **hydrogen** (resulting from carbohydrate fermentation by anaerobic bacteria), **ethanol** (endogenous, originated from microbial fermentation of carbohydrates in the gastro-intestinal tract, and exogenous, deriving from alcoholic drink), **ammonia** (another component present in tobacco fumes), **carbon dioxide** (which production can be considered as a measure of metabolism), **oxygen**.

The hardware design of the Wize Sniffer 1.0. is described, as well as the software design. About the hardware design, we took inspiration from a study, conducted by D. Guo et al. [1], whose aim was to develop a new specific breath analysis system making use of chemical sensors that were particularly sensitive to the biomarkers and compositions in human breath. They used metal oxide semiconductor gas sensors, manufactured by Figaro Enginnering. Being these sensors very robust, sensitive, resistant to humidity and ageing, easy to read, we retained very reasonable using this kind of sensors.

The box embedding the sensors was designed in collaboration with COSMED s.r.l. Its general scheme had a gas store bag/box, an element whose task was to absorb the water vapor from the breath, and a disposable mouthpiece. The gas sensors, placed in the store chamber, sense gas particles and generate measurable electronic signals. The data from the sensors are subsequently sent to the computer by means of TCP/IP trasmission protocol and saved for further analysis.

In order to evaluate Wize Sniffer 1.0.'s performances and make it ready to be integrated in the "Wize Mirror", we drafted a measuring protocol to test our device on a population containing individuals of different age, habits, body type.

In the conclusive section of this work, the test results are discussed, as well as the advantages and the limits of our device compared with the traditional breath analysis techniques. A "to do list", for Wize Sniffer future developments, is drafted, in order to improve its performances.

Indeed, in order to obtain higher sensitivity, the integration of polyaniline electrospun nanofibers as sensors' sensing element, will be the next great challenge.

Chapter 1

Design of the portable device: Wize Sniffer 1.0

1.1 Aims of the work and SEMEOTICONS Project

The **Wize Sniffer (WS)** is a new **portable** device for breath analysis in home care. The device is designed to sense only an effective number of substances, giving informations on the possible **state of well-being** of an individual, as provided in **SEMEOTICONS (SEMEiotic Oriented Technology for Individual's CardiOmetabolic risk self-assessmeNt and Self-monitoring)** European Project (www.semeoticons.eu) and described in [2], Proceedings of SUPERHEAL2014 conference, Angers, France.
By the Wize Sniffer we intended a **hardware/software tool for both the analysis of volatile organic compounds of breath and a platform for data mining and data integration**, being able to provide useful information about the "breathprint", the analog of fingerprint for the state of health

of an individual.
Usually gases are identified as biomarkers using instrumentations such as gas chromatography (GC),very accurate, or electronic nose (e-nose). Nevertheless, Gas Chromatography is expensive, time consuming and non portable. E-nose has the advantages of low-cost and easy operation, but is not particularly useful for analyzing more than one breath substance. As a consequence, in recent years, the necessity to develop a portable device for breath analysis, **easy to use**, able to make **real-time measures**, feasible for patients living far from medical structures or which could be integrated with other device, as in SEMEOTICONS European Project.
The idea behind SEMEOTICONS Project is to exploit human face as an indicator of individual's health status and translate the semeiotic code of the face into measurements and descriptors automatically evaluated by a computerized application. In particular, the eligible application field is the one of cardio-metabolic diseases **prevention**, for which healthcare systems are registering an exponential growth of social costs, especially due to expensive diagnostic resources, often improperly prescribed. One of the principal aims of SEMEOTICONS Project is to move the semeiotic analysis from the office of medical doctors closer to individual's normal-life settings and enable normal people to self-assess their personal well-being status, particularly concerning their cardio-metabolic risk.
Cardio-metabolic risk factors include **obesity**, physical inactivity, **smoke**, **alcohol abuse**, **abnormal lipid metabolism**, hyper-glycaemia and **arterial hypertension**. Educational programs and lifestyle interventions represent effective tools for reducing cardio-metabolic risk profile.
To this end, SEMEOTICONS will design and construct an innovative multisensory system integrated into a hardware platform having the exterior

aspect of a mirror: the so-called "**Wi**se Wi**z**ard Mirror" or "**Wize Mirror**"(see Figure 1.1). This will easily fit into **users' home or other sites of their daily life** (fitness and nutritional centres, pharmacies, schools and so on).

The Wize Mirror will collect data mainly in the form of videos and images from multispectral cameras and other advanced detection tools, and **gas concentration** signals from the WS.

This work has been addressed to design a **first prototype**, so called **Wize Sniffer 1.0.**, of a portable device able to detect those breath compounds related to the cardio-metabolisk risk as well as to noxious habits (such as carbon monoxide, hydrogen, ethanol, carbon dioxide, oxygen).

The integration of the data from the WS 1.0. with the ones deriving from the Wize Mirror (state of nutrition, mental condition, states affecting the colour or the appearance of the skin such as anemia, or presence of products of glucose and lipid metabolism) will provide a Virtual Individual's Model, which will be used to compute and trace the daily evolution of an individual's "**wellness index**". A well-being diary based on this index will enable users to evaluate and personally correlate the lifestyle to their wellness and health status.

1.2 Detected volatile organic compounds (VOCs)

It should be noted that does not exist a list of precise exhaled substances which can be considered as indices of well-being, rather some VOCs can be taken into account when it talks about well-being correlated to cardio-metabolic risk.

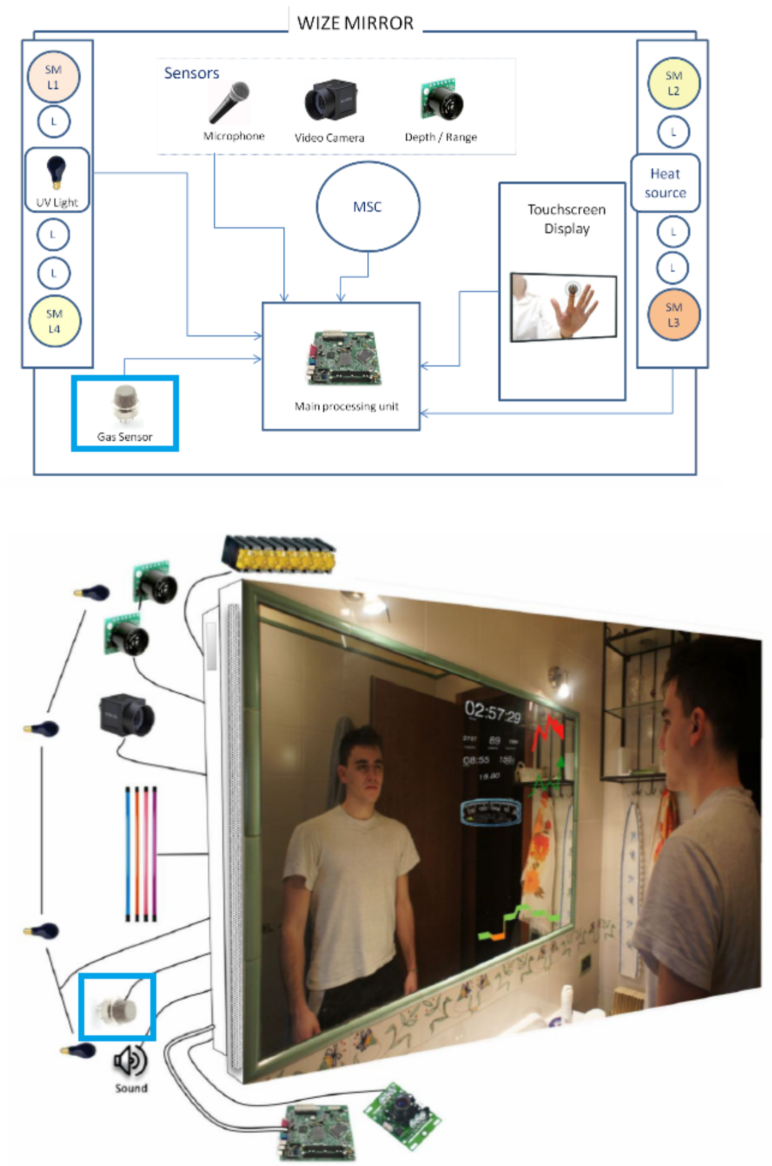

Figure 1.1: **SEMEOTICONS European Project- On the top, a schematic representation of the Wize Mirror suite of sensors, processing unit, display and accessories. SMLi(i=1,...,4) are multicolour lights used to explore photometric 3D reconstruction. On the bottom, the Mirror appearance: the user will be only aware of the touch-screen, of the lights, which will appear as normal lights, and of the gas sensors, called *Wize Sniffer*, which will have a "protrusion" to better "sniff" user's breath.**

As a consequence, **we considered worthwhile detecting those compounds linked to alcohol intake, cigarette smoke, oxidative and metabolic stress**, always taking into account that, in some cases, a breath a biomarker may be indicative of about more than one disease or metabolic-disorder; in other cases, one particular disease or metabolic disorder can be characterized by more than one chemical species. Then, the molecules which our attention has been focused on:

- **Oxygen (O_2)**

  Exhaled air has a decreased amount of oxygen and an increased amount of carbon dioxide. These amounts show how much oxygen is retained within the body for use by the cells and how much carbon dioxide is produced as a by-product of cellular metabolism.
  Exhaled oxygen amount is about 13.6%-16%. Lower values may be due to respiration disorders.
  Not only, it is weel known that, in smokers, carbon monoxide molecules take the place of oxygen in red blood cells, reducing the amounts of oxygen in blood and in exhaled gas.

- **Carbon dioxide (CO_2)**

  Mean carbon dioxide concentration in exhaled breath is about 4% (= 40000ppm).
  It is produced as a waste product when energy is released during certain metabolic reactions of cellular respiration.
  In healthy individuals, partial pressure in arterial blood is very close to the partial pressure in expired gases.
  So, carbon dioxide production can be considered as a measure of metabolism.
  Not only, it can be considered a good index for oxidative stress. Indeed,

among all the reactions present within cellular respiration there is the oxidative phosphorylation that produces not only carbon dioxide, but also reactive oxygen species such as superoxide and hydrogen peroxide. This lead to propagation of free radicals, which can damage cells and contribute to the onset of many disease.
The increase of CO_2 can be due also to physical activity, for example. There is a decrease in presence of some forms of congenital heart disease (for example, cyanotic lesions that result in a bluish-grey discoloration of the skin and in a lack of O_2 in the body).
Also individual's breathing rate influences the level of CO_2 in blood and, as a consequence, in exhaled gas. Breathing that is too slow causes respiratory acidosis (that results in an increase of CO_2 partial pressure in blood, which may cause hypertension, build up of heart rate), while breathing that is too rapid leads to hyperventilation, which may cause respiratory alkalosis (that results in decrease of CO_2 in blood; so, it can no longer fulfill its role of vasodilator, resulting in arrhythmias, extra systoles).
Carbon dioxide is also one of constituents of tobacco smoke (it is present in 13%).

- **Carbon monoxide (CO)**
  Mean carbon monoxide concentration in exhaled breath is about 3,5 ppm. Naturally, carbon monoxide presents in human breath is related to the one present in blood. CO acts as a cellular messenger, as a promoter of neurovascular growth (it's not always beneficial, since it plays a bad role in tumor growth) and functions in vasodilation. Because of these carbon monoxide's roles in the body, abnormalities in its metabolism

have been linked to a variety of diseases, including neurodegenerations, hypertension, heart failure, and inflammation.

Carbon monoxide is also absorbed through breathing and enters the blood stream through gas exchange in the lungs.

Normal circulating levels in the blood are 0% to 3%, and are higher in smokers (who exhale until 10-30 ppm of carbon monoxide). Indeed, carbon monoxide is the major component present in tobacco fumes (75,95%).

So, increasing levels of exhaled carbon monoxide can be detected in smoking subjects: 13.8 - 29 ppm.

- **Hydrogen (H_2)**

  The passage of unabsorbed dietary carbohydrate into the caecum is followed in most individuals by the production of hydrogen, a metabolic consequence of carbohydrate fermentation by anaerobic bacteria. This hydrogen traverses the gut wall, and is transported via the circulation to the lungs, where is excrated in exhaled breath.

  Not only, a certain amount of exhaled hydrogen is the result of fermentation by oropharingeal bacteria.

  As a consequence, increased values of breath hydrogen may be due to overweight problems, intestinal diseases, improper life-style.

  A rise in exhaled hydrogen values occurs while/after smoking. A decrease occurs after exercise and in case of hyperventilation, which reduce the concentration of exhaled breath hydrogen by ”washing out” it from the lungs. About the quantities, for hydrogen the baseline value is about 9.1ppm.

- **Ethanol (C_2H_5OH)**

  Endogenous ethanol (whose values are very low: 0.62ppm) is originated from microbial fermentation of carbohydrates in the gastro-intestinal tract. Then, it enters the blood. As a consequence, it may increase in exhaled gas mixtures because of alcoholic fermentation of an excessive over-load of carbohydrates.

  About the exogenous one, it comes from alcoholic drink.

  Ethanol is one of the causes of accumulation of free radicals into the cells, that can result in oxidative stress, causing arrhythmias and depresses the contractility of cardiac muscle.

- **Ammonia (NH_3)**

  Mean baseline levels of ammonia in exhaled gas are about 0.42ppm. Elevated breath ammonia usually could be due to liver disease, such also to kidney disease.

  Nevertheless, ammonia is also one of the three major compounds, together with nitric oxide and carbon monoxide, of tobacco fumes (it is present in approximately 22,15%). All these three gases can have detrimental effects on the respiratory system. About ammonia, it forms a strong alkaline solution with water. When inhaled, ammonia irritates the upper respiratory tract including the nasal cavity, pharynx, larynx and trachea. Irritation results in coughing and hoarseness.

1.3 Wize Sniffer 1.0 - Hardware

1.3.1 Figaro and COSMED gas sensors, and temperature and humidity sensor

The choice of gas sensors has been made taking into account:

- the breath compounds to detect: oxygen, carbon dioxide, carbon monoxide, ammonia, hydrogen,ethanol;
- the working principle of the sensors.

Furthermore, a useful guidance in the choice of the type of gas sensors has come from a study conducted by D. Guo et al.[1], whose aim was to develop a new specific breath analysis system making use of chemical sensors that were particularly sensitive to the biomarkers and compositions in human breath. For this purpose, they used **metal oxide semiconductor gas sensors**, manufactured by **Figaro Enginnering**.
Considering this type of sensors easy to use, robust, sensitive, resistant to humidity and ageing, we have ordered from Technosens 6 Figaro gas sensors, listed in table1.1 Their general structure is described in Appendix A, while an example of their basic measuring circuit is shown in Fig.1.2.

A voltage (5V DC) is applied across the sensor element which has a resistance (Rs) between the sensor's two electrodes and a load resistor (RL) connected in series. When the sensors sense gas molecules, a change in Rs occurs, that is measured indirectly taking the voltage value across the load resistance V_{RL}:

$$Rs = RL\frac{Vc - V_{RL}}{V_{RL}} \tag{1.1}$$

Since sensors's electrical conductance and selectivity strongly depend on change in temperature (as shown in Fig.1.3), a further voltage (5V DC)

Table 1.1: **Figaro gas sensors used for the WS 1.0..**

Figaro gas sensor	**Detected molecule(s)**	**Optimal detection range**
TGS2602	hydrogen, ammonia, ethanol, hydrogen sulfide, toluene	1-10ppm
TGS2620	hydrogen, carbon monoxide, ethanol, methane, isobutane	50-5000ppm
TGS4161	carbon dioxide	350-10000ppm
TGS2442	carbon monoxide	10-1000ppm
TGS2444	ammonia	10-100ppm
TGS821	hydrogen	1-1000ppm

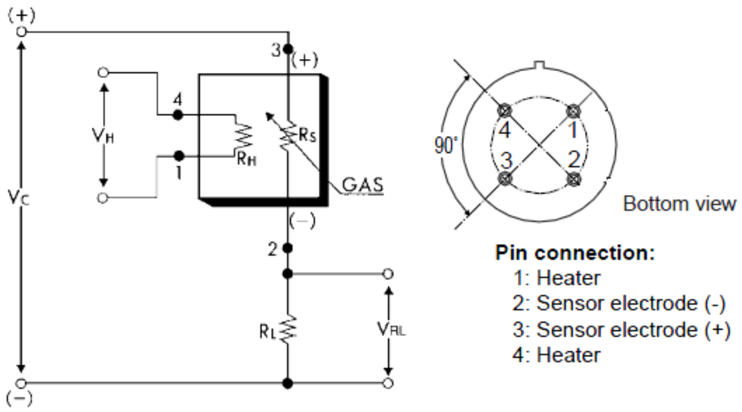

Figure 1.2: **Basic measuring circuit for TGS2620 and TGS2602**

is applied across remaining two pins to heat the sensing material, and maintain the sensor at a fixed temperature.

Also sensor/water vapour interaction leads to an increase in the sensor's electrical conductance and to a lack of selectivity. That's why a sensor for temperature and humidity (Sensirion SHT11, see Appendix A) has been placed within our device, in order to monitor and mantain constant test conditions.

An example of sensitivity characteristics of these sensors is shown in Figure 1.4. In the presence of a detectable gas, the sensor's conductivity increases depending on the gas concentration in the air. The sensors response

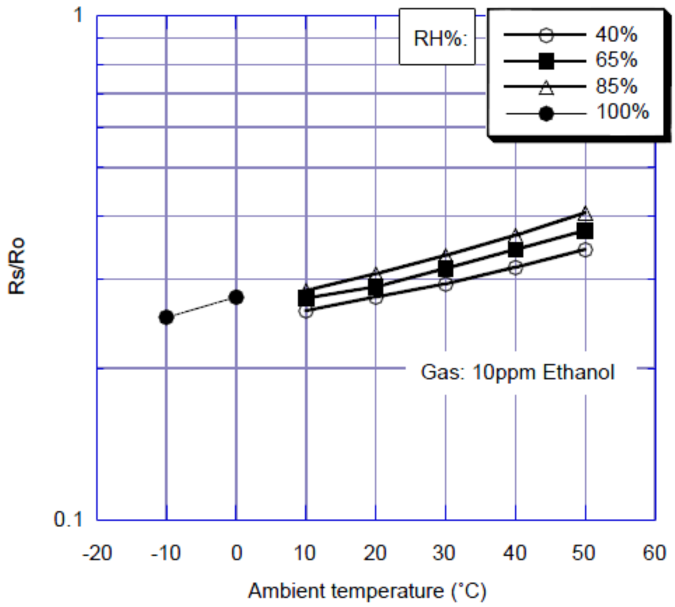

Figure 1.3: **TGS2602 temperature and humidity dependency characteristics. Sensor/water vapour interaction leads to an increase in the sensor's electrical conductance and to a lack of selectivity. Same effects occur when temperature increases.**The Y-axis shows the ratio of sensor resistance in 10ppm of ethanol under various atmospheric conditions (Rs) to the sensor resistance in clean air under the same atmospheric conditions (Ro)

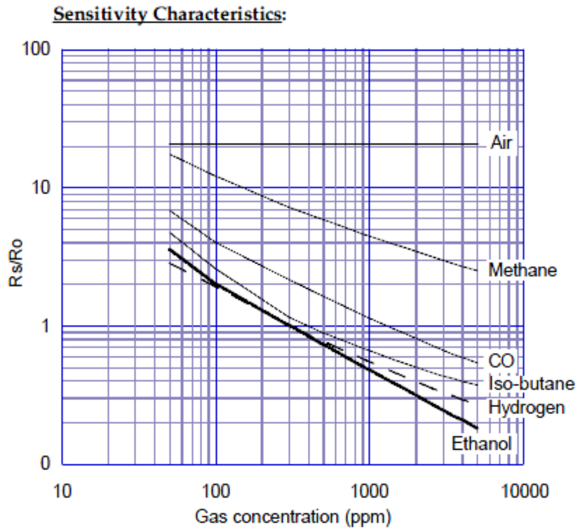

Figure 1.4: **TGS2620 sensitivity characteristic in bilogarithmic scale.** The Y-axis is indicated as sensor resistance ratio between Rs (= Sensor resistance in displayed gases at various concentrations)and Ro (= Sensor resistance in 300ppm of ethanol)

time are lower than 20-30sec.

It should be noted that among the substances detected by Figaro sensors, there are also molecules, such as methane, toluene, isobutane, hydrogen sulfide, which we were not interested to.

However, as shown in the sensitivity characteristics, the detectable range for methane, toluene, isobutane are higher than the quantities (on the order of ppb) present in exhaled gas. As a consequence, these molecules will not be detected.

On the contrary, the detectable range for hydrogen sulfide goes from 0.1ppm to about 1ppm, so we can say that TGS2602 is able to detect the amounts of hydrogen sulfide in our breath (about 0.33ppm). Hydrogen sulfide is usually associated with halitosis, which origin may be due to oral cavity or other sources (see Chapter ??). Furthermore, it is also considered as a vascular relaxant agent, so we retained useful its detection, too.

All Figaro gas sensors has been placed in a store chamber, where exhaled gases are gathered, while other two gas sensors, manufactured by **COSMED s.r.l.**, one sensitive to oxygen and the other one to carbon dioxide, has been placed outside the store chamber in order to make them work in flow (this aspect will be discussed in depth in the following section).
About these sensors' outputs, COSMED s.r.l. reported us the informations shown in table 1.2.

Table 1.2: **COSMED gas sensors used for the WS 1.0.**. Sensors' warm-up time: 30min (for both sensors)

	sensors output
Oxygen sensor	from 0 to 4V+/-0.01V @20.93% O_2
Carbon dioxide sensor	0.02V +/-0.01V @ 0.03%
	0.67V +/-0.01V @ 0.70%
	1.75V +/-0.01V @ 2.00%
	3.5V +/-0.01V @ 5.00%

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1.3.2 The circuit and Arduino Ethernet board

In order to receive the data from the sensors, we decided to make use of a microcontroller board, that is Arduino. The choice of the type of Arduino board has been made taking into account:

- Number of bits of analogue-to-digital converter;
- Number of analogue input pins;
- Possibility to use an Ethernet module

The latter requirement was due to the advantage of sending the data from Arduino to computer wireless. Our aim was to create a Telnet server by us-

Figure 1.5: **Arduino Ethernet has 14 digital input/output pins (but 4 of them are reserved for interfacing with Ethernet module, so the number of available pins becomes 9); 4 of the digital pins are available as PWM outputs. It has 6 analogue input pins, each of which provides 10 bit of resolution (=1024 different values). Recommended input voltage is 7-12V.**

ing Arduino, in order to read data by making a simple request to the server itself from our computer. These aspects will be described in detail in the section referring to the software- data communication protocol.
Basing on such requirements, Arduino Ethernet board (shown in Figure 1.5) has been choosen.

After choosing the microcontroller board, we have realized the electronic circuit.
In Figure 1.6 is shown the circuit (front),a scheme of it is reported in Figure 1.7 and finally, in Figure 1.8, is shown the circuit board connected to Arduino Ethernet.

1.3.3 The box embedding the sensors

The box embedding the sensors has been realized in collaboration with COSMED s.r.l.
A guidance has still come from the study, mentioned before, conducted by

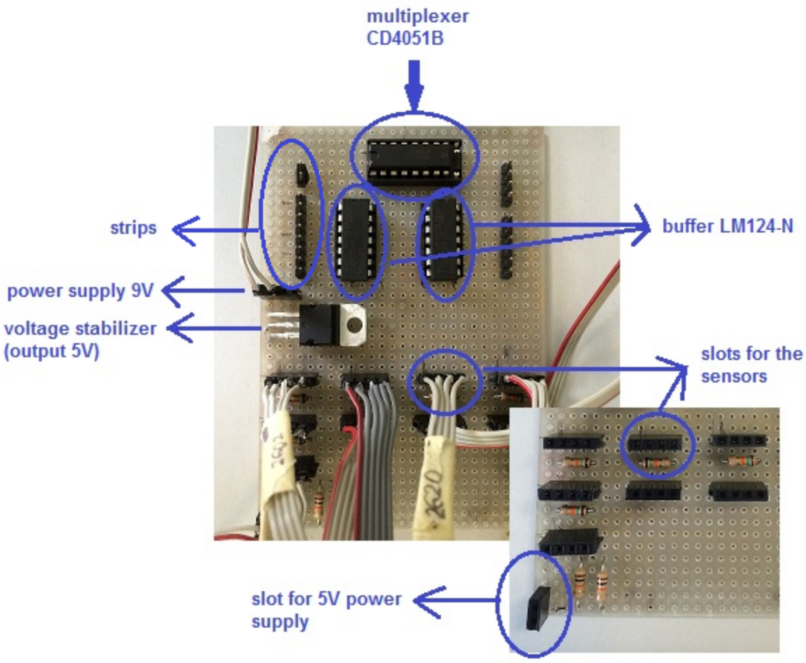

Figure 1.6: **Circuit board (front)**.The sensors were: 6 Figaro gas sensors + 2 COSMED gas sensors + Sensirion temperature and humidity sensor, that is 9 outputs to be read. Having Arduino Ethernet board 6 analog input pins, it was necessary to integrate a multiplexer (CD4051B, Texas Instrument), in order to switch the reading output as need be. A scheme of CD4051B is shown in Appendix A. 8 buffers (LM124-N, Texas Instrument which scheme is shown in Appendix A) were integrated between sensors' output and Arduino analogue pins in order to stabilize the voltages output from the gas sensors. The slots for the sensors had necessary connections for voltage supply, heater voltage (in case of Figaro sensors), output and GND. The power circuit and the heater circuit were separated, because the second required more current than the first and overloaded the power supply. So, for the power circuit a power supply of 9V and a voltage stabilizer with an output of 5V have been used, while for the heater circuit a power supply of 5V has been used.

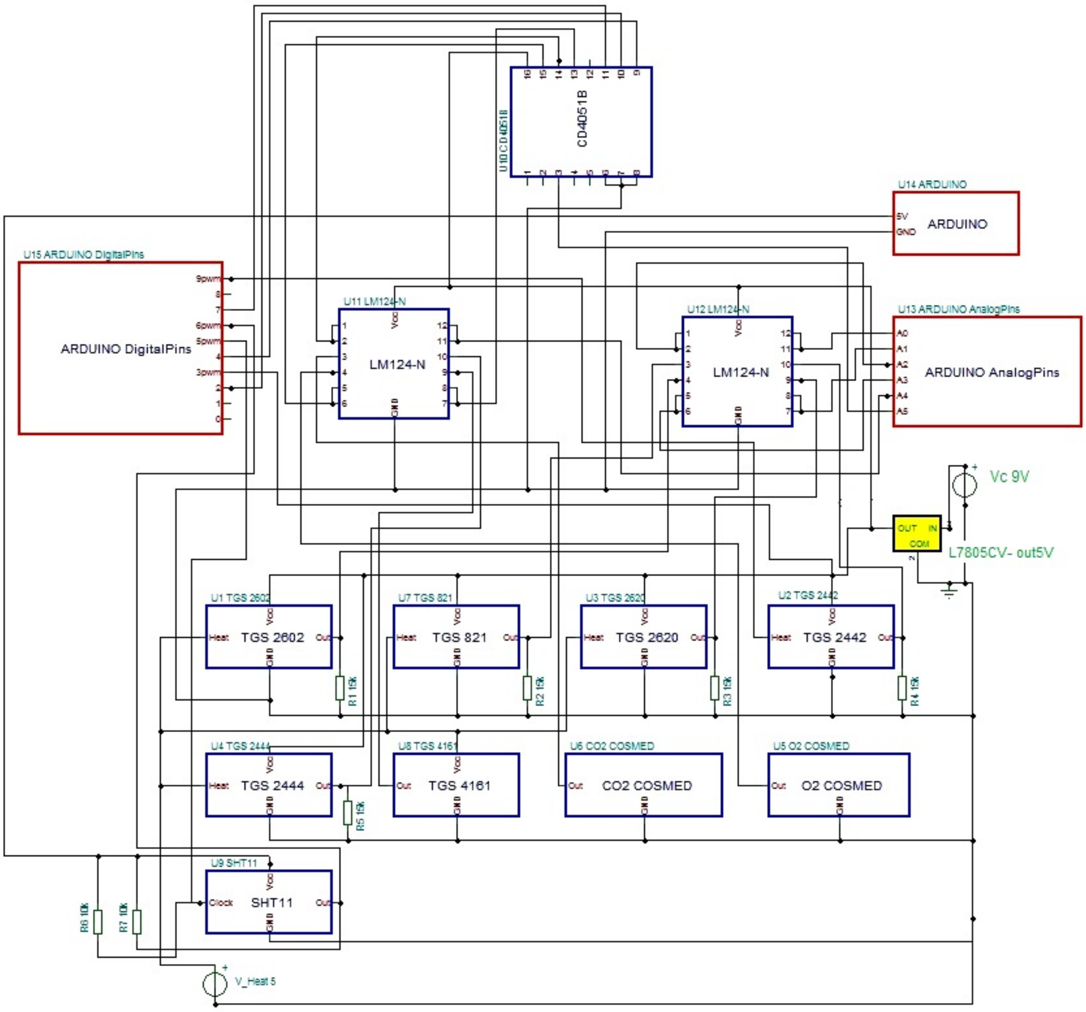

Figure 1.7: **General scheme of the circuit realized for the Wize Sniffer 1.0.** Load resistance RL, equals to 15KΩ, has been used for Figaro sensors' measuring circuit(see 1.2). Two resistance equal to 10KΩ has been used as pull-up resistance in order to stabilize the clock and the output of Sensirion SHT11 (see Appendix A). In the case of TGS2442, its voltage pin and heater pin were linked to Arduino digital pin 3 and 9 (both available as PWM outputs), respectively. This choice we made was due to the need for a heating/powering cycle (see Appendix A). The sensors manufactured by COSMED s.r.l. are powered by the WS.

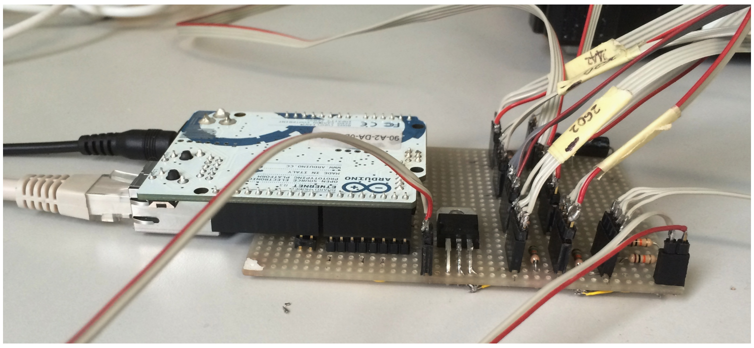

Figure 1.8: **Circuit board connected to Arduino Ethernet**

Figure 1.9: **First WS 1.0 general idea- collecting exhaled air in a store chamber**

Dongmin Guo et al. [1], where a system like the one shown in Figure 1.9 has been described. This system operated in three phases: gas collection, gas sampling, and data analysis. It had a gas sampling bag/box (A), an element (B) whose task was to absorb the water vapor from the breath, and a disposable mouthpiece (C). The gas sensors sensed gas particles and generated measurable electronic signals, subsequently sent to the computer for feature extraction, pattern analysis and classification.

Grounding on this scheme, we thought about a block diagram of our device like the one shown in Figure 1.10. We provided for a straw, necessary to convey the exhaled gas toward the box, and for a humidity filter, made of hygroscopic material, to absorb the water vapor from the breath.

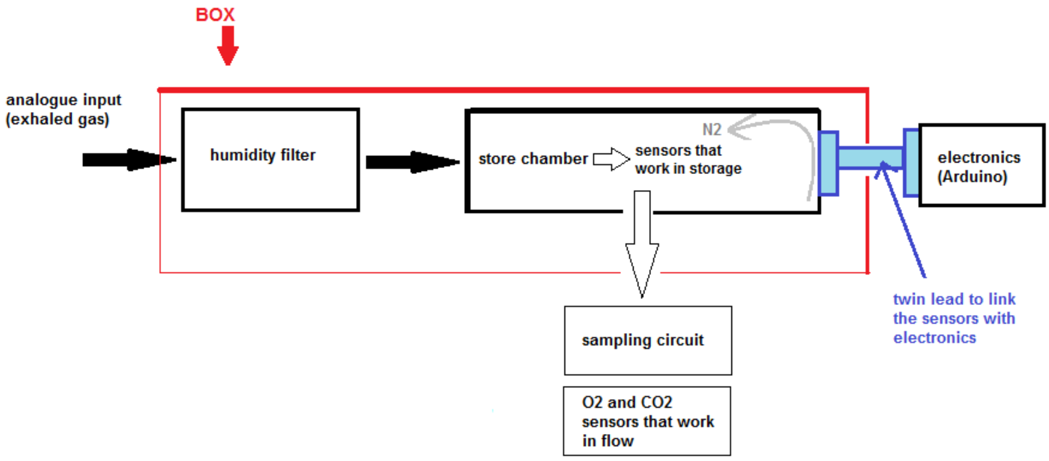

Figure 1.10: **A first scheme of our device, made in collaboration with COSMED s.r.l.**. The presence of two sensors, one for oxygen and one for carbon dioxide, workin in flow, was considered useful to compare the values output from these sensors with the ones output from the sensors working in store.

The store chamber's dimension was fixed at about 600ml, according to pulmonary capacity.

A sampling circuit was considered useful to inject, at a certain rate (=120 ml/sec, basing on [1]), the exhaled gas samples in a second chamber, where two COSMED gas sensors (one sensitive to oxygen, the other sensitive to carbon dioxide, both working in flow) were placed.

We provided also for a pump to purge the chamber and recovery the sensors' steady state.

A kind of twin lead was included to link the gas sensors with other electronics, and to sent the collected data to Arduino board, then, to the computer.

An important aspect we considered, was that the chamber embedding the sensors could be opened easily, in order to be able to change the sensors as need be.

Figure 1.11: **The corrugate tube (made of PVC) with HME filter**

Subsequently, the first prototype has been manufactured, so called "**Wize Sniffer 1.0**".
Let's see in detail how the WS 1.0 is made.
A corrugate tube (see Figure 1.11) allows the exhaled gas from the individual to reach the gases store chamber. Indeed, the individual breaths in a disposable mouthpiece placed at the beginning of tube. A heat and exchange moisturizers (HME) filter retains part of the humidity present in human exhaled breath.

Then, the gases reach the store chamber (which dimensions equal about 600ml as pulmonary capacity), where the six gas sensors (the commercial ones, manufactured by Figaro Engineering and described in precious section) are placed. They are exposed, within the store chamber, upside down, so that their sensing part (their "head"), can sense gases' molecules. Two uni-directional valves allow the gases to enter the chamber and to be collected within it, preventing the mixing with ambient air. In Figure 1.12 is shown a store chamber's design detail, while in Figure 1.13 is shown the store chamber with the gas sensors placed within it. The gas sensors sense gases' particles and generate an electric measurable signals (it means, a voltage), which is sent, by twin leads (as shown in Figure 1.13), to Arduino Ethernet

Figure 1.12: **A detail of the store chamber's design**

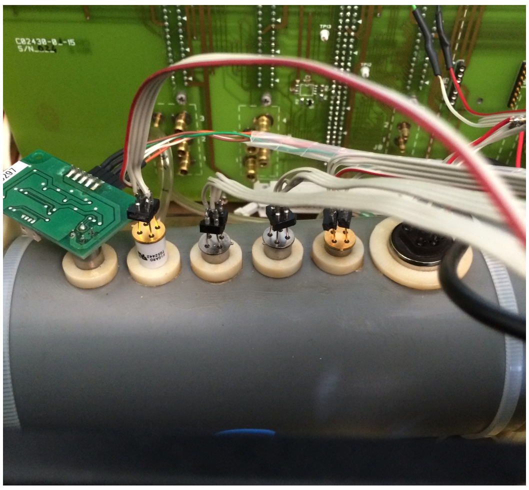

Figure 1.13: **All the Figaro gas sensors are placed with their "head"(which corresponds to the sensing part) in the store chamber where the ehaled air is gathered.** The materials used for the store chamber were PVC and ABS.

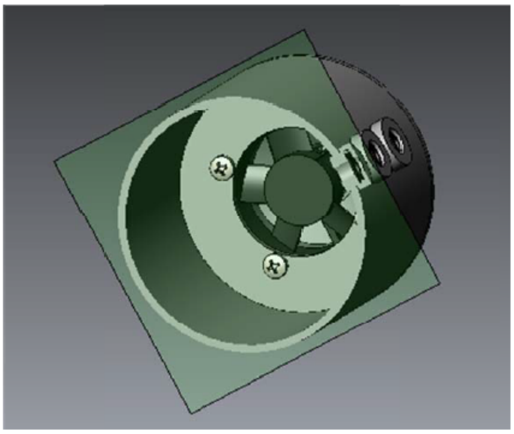

Figure 1.14: **A fan allows to purge the store chamber after each measuring.**

board.
The store chamber is provided also with a flushing pump (see Figure 1.14) which allows to purge the chamber itself, after each measuring.

The chamber is placed in a box (which dimensions are: 30cm x 38cm x 14cm) shown inFigure 1.15. The reverse side of the box is shown in Figure 1.16.

The two sensors, manufactured by COSMED s.r.l., one sensitive to oxygen, one sensitive to carbon dioxide, work in flow: they sense gases' stream that is injected at a constant rate (120ml/sec) by the sampling pump. This electromechanic pump takes gas samples from the store chamber and injects them where the O2 and CO2 sensors are placed. Then, gases are brought back to store chamber.
The final configuration of our "Wize Sniffer 1.0" is shown in Figure 1.18, while in Fig.**??** is shown an example of its employment.

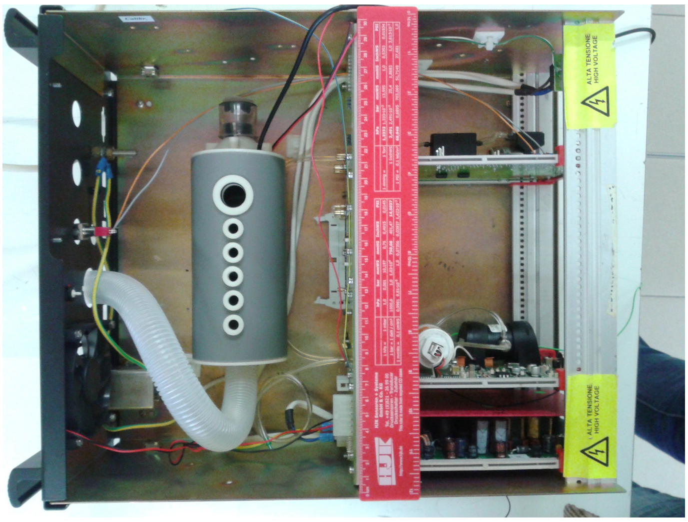

Figure 1.15: **The store chamber is placed in a box as above.**

Figure 1.16: **The reverse side of the device. The COSMEd sensors for oxygen and carbon dioxide can be seen. On the right, the sampling pump which allows these two sensors to work in flow.**

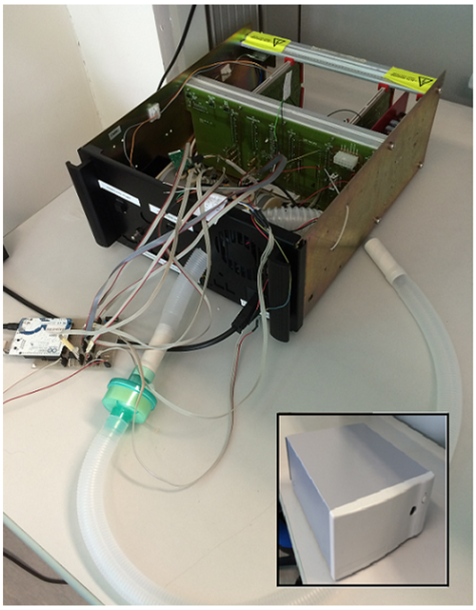

Figure 1.17: **Wize Sniffer 1.0. It requires a voltage supply of 12V.**

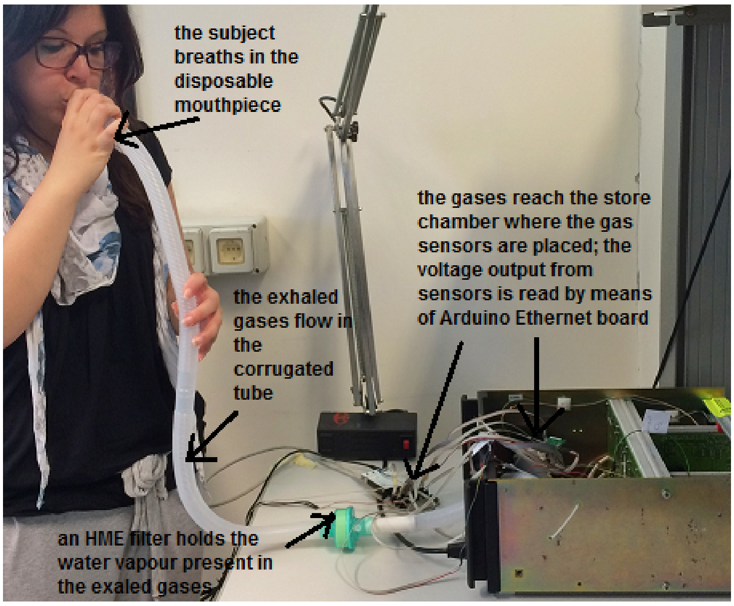

Figure 1.18: **The individual breaths in a disposable mouthpiece; the gases, by means of a corrugated tube, reachs the store chamber where the gas sensors are placed. The output voltages are then read by means of Arduino Ethernet board and sent to pc for further analysis.**

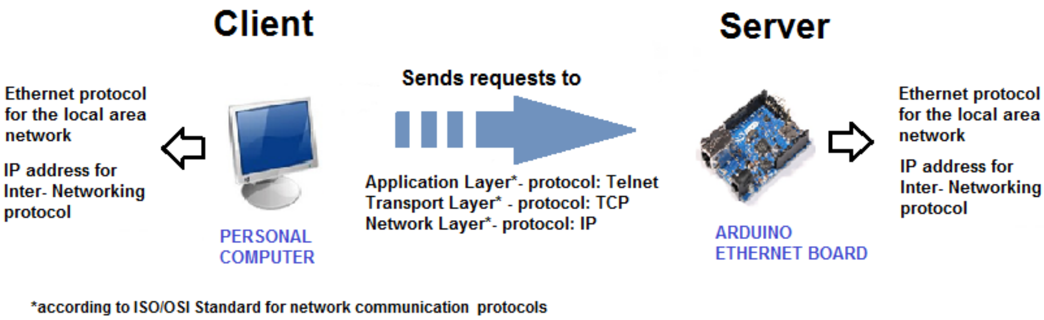

Figure 1.19: **A simple description of the communication protocol used to allow the data exchange from Arduino to personal computer.**

1.4 Wize Sniffer 1.0 - Software: how to communicate with Arduino board?

Usually, when an Arduino board is used to read data from sensors, or from other devices, a USB cable is the most simple way to communicate a personal computer with Arduino itself. Nevertheless, this is not very advantageous if the computer is placed far away from Arduino.

A wireless connection between the devices becomes necessary; our idea was sending the data from Arduino to computer wireless. Our aim was to create a Telnet server by using Arduino, in order to read data by making a simple request to the server itself from our computer.

The idea we had can be described graphically as in Figure 1.19.

First of all, we programmed Arduino Ethernet board in order to create a Telnet Server. Telnet is a client-server protocol, based on TCP protocol, pertaining to application layer, according to ISO/OSI Standard for Network Communication Protocols. Usually, a client makes a connection to server's port 23.

The pseudocode of the program implemented for Arduino Ethernet board to create a Telnet Server and receive data from it is the following:

- ✓ Uploading Ethernet libraries, network configuration, setup() function
  - ❖ *<Ethernet.h>*, Arduino mac Address, Arduino IP Address
  - ❖ *EthernetServer server = EthernetServer(23); // telnet defaults to port 23*
  - ❖ *Ethernet.begin (mac, ip); // initialize the ethernet device*
  - ❖ *server.begin(); // start listening for clients*
- ✓ *Loop() { if (client==true)*, there will be bytes available to read:
  - ❖ *out(i) = analogRead(i)*0.0049; // reading Analogue Pin values*
  - ❖ *client.print(out(i)); // print of the 8 sensors' values (Volts)*

Figure 1.20: **The pseudocode of the program implemented for Arduino Ethernet board to create a Telnet Server and receive data from it.**

Libraries for Ethernet connection were included, all the parameters necessaries to network configurations (for example, Arduino IP address and MAC address) were inserted, two variables "server" (of "EthernetServer" class) and "client"(of "EthernetClient" class) were created in order to set up the connection mode and to give the server instructions about the data to transmit.

Furthermore, libraries for reading temperature and humidity sensor Sensirion STH11 were included, "float" variables were defined in order to read data from each sensor, some Arduino's digital pins were dedicated to multiplexer control input while other two (available as PWM outputs) were chosen to set up the heating/powering cycle for TGS2442. The pins were setted to a "HIGH" value or "LOW" value according to the cycle wave (see Appendix A).

To send a request to the Telnet Server, and receive data from it, we created a TCP/IP object implemented on Matlab. It allows to make a connection to server's port 23 and fill the buffer (which dimensions can be set) with the data coming from the server. It means, the buffer is filled with the sensors' values array. Moreover, the data received can be saved in a text file in order to be managed for further analysis.

The pseudocode of the program implemented on Matlab is the following:

- ✓ Opening connection and reading a "tcp/ip object"
  - ❖ *t = tcpip('Arduino IP Address', 23);* *// connect to (port 23) Arduino*
  - ❖ setting (t, Buffer dimension, other properties);
  - ❖ *fopen(t) ;* *// start connection*
  - ❖ Until there will be 'BytesAvailable' - -> *A= fscanf(t)*
- ✓ Saving the data
  - ❖ *fprintf (fileID, A);* *// writing the data read in a file .txt*
- ✓ Closing connection
  - ❖ *fclose(t);*

Figure 1.21: **Send a request to the Telnet Server by means of TCP/IP object implemented on Matlab**

Chapter 2

Data acquisition

After having developed this first prototype of our ”Wize Sniffer”, the next step was to test it in order to evaluate its performances and make it ready to be integrated in the ”Wize Mirror”.
For this purpose, first we tested each gas sensor singularly, making them sense the several substances (on the order of picoliter) they are sensitive to; then, a measuring protocol was draft in order to test the ”Wize Sniffer 1.0.” on a population containing individuals of different age, habits, body type.

2.1 Single tests

The only aim of the tests was to evaluate sensors'response on a quality level. That is, only evaluate their change, their rise in voltage outputs when sensing that/those gas/gases they are sensitive to.
During this first phase of the work we restricted our efforts to evaluate each sensors' functioning on a quality level, because a new calibration, that is, evaluate voltage outputs in response to precise substances' concentrations on

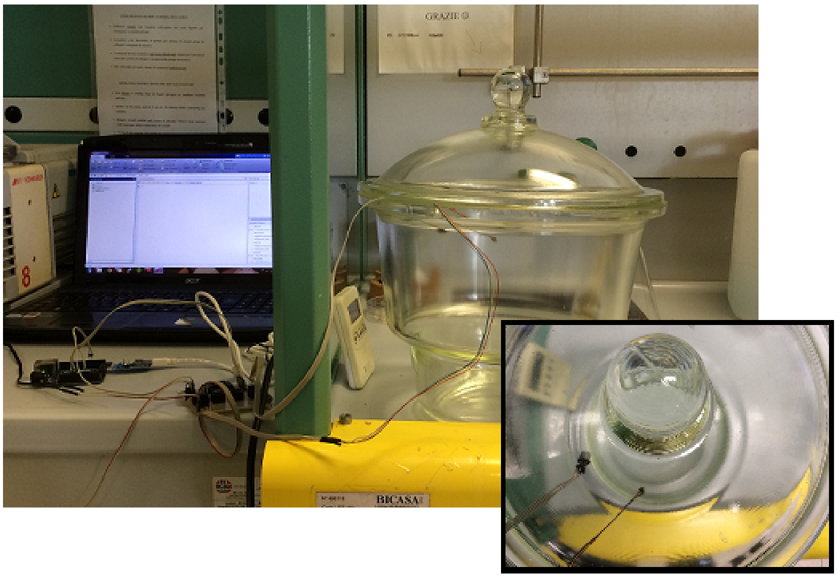

Figure 2.1: **Sperimental set-up at Department of Chemistry and Industrial Chemistry of University of Pisa- On the right, the desiccator, where Figaro gas sensor and temperature and humidity sensor Sensirion SHT11 (necessary to check the temperature and humidity within the contenitor) were placed.**

the order of parts per million, would have required greater efforts in terms of instrumentations and equipment.

Among the tests conducted on each sensor singularly, here are described the ones conducted on **TGS2602** and **TGS2620** (which are the two "multi-sensitive" sensors) at the **Department of Chemistry and Industrial Chemistry of University of Pisa**, in collaboration with Dr. Federica Chiellini and Dr. Dario Puppi.

Each sensor was placed, toghether with the temperature and humidity sensor (Sensirion SHT11) in a airtight desiccator whith a diameter of about 20cm, as shown in Fig.2.1.

The substances (which the sensors are sensitive to), were placed, one by one, on the bottom of the desiccator; they were on the order of picoliter, as

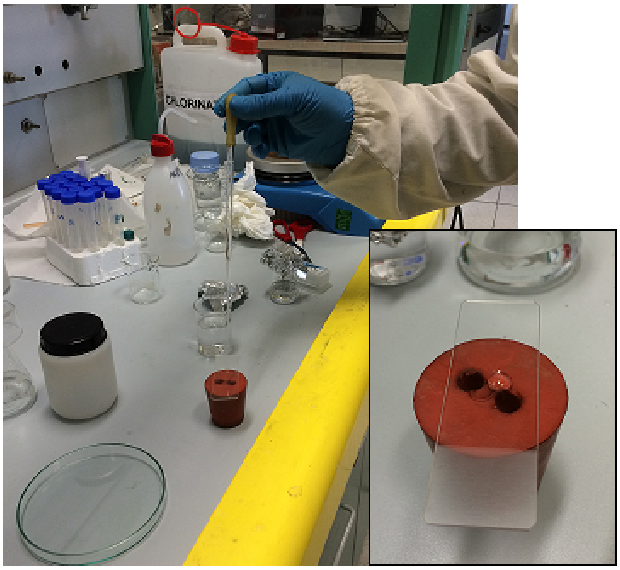

Figure 2.2: **Each substance, which the sensor under test is sensitive to, was taken on the order of picoliter (the quantity as minimum as possible) and placed on the bottomof desiccator.**

shown in Fig.2.2. The data from sensors were registered and saved by means of a personal computer, as explained in previous chapter (see also Fig.2.1). For TGS2602 (sensitive to hydrogen, ammonia, ethanol, hydrogen sulfide, toluene) pico-liters of ethanol, ammonia, toluene were placed on the bottom of desiccator, one by one. It was not possible to involve hydrogen and hydrogen sulfide in the tests. The voltage outputs are shown in Fig.2.3. It should be noted how the voltage values, in response to ethanol and toluene, rose faster than in response to ammonia. Similarly, the peak values relative to ethanol and toluene were higher than the one corresponding to ammonia. This is in line with the slopes of bilogarithmic curves mapping the sensor sensitivity characteristics for the several substances. Indeed, TGS2602 sensitivity characteristic for ethanol shows a slope $\alpha_{TGS2602_{Eth}}$ equals to -0.416939 (just as for toluene, $\alpha_{TGS2602_{Tol}}$ equals to -0.564573), whereas for ammonia

$\alpha_{TGS2602_{NH_3}}$ is -0.28658.

For TGS2620 (sensitive to hydrogen, methane, ethanol, isobutane, car-

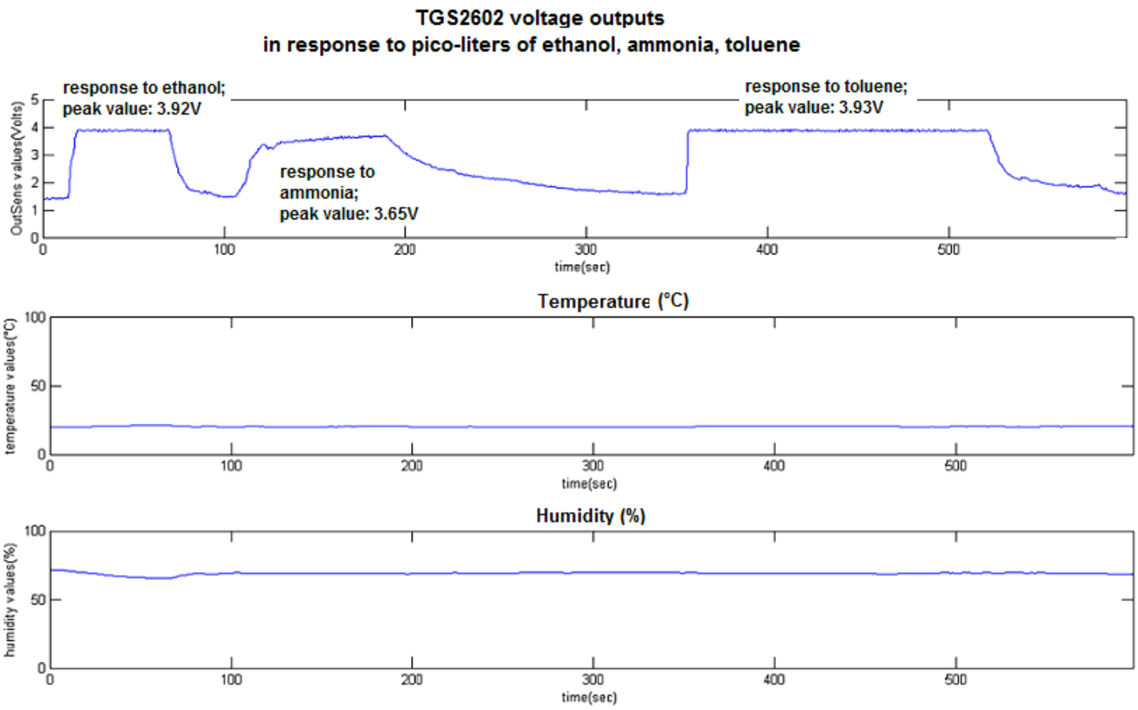

Figure 2.3: **TGS2602 outputs in response to ethanol, ammonia, toluene concentrations on the order of pico-liters.**It should be noted how the voltage values in response to ethanol and toluene rose faster than in response to ammonia. Similarly, the peak values relative to ethanol and toluene were higher than the one corresponding to ammonia. This is in line with the slopes of bilogarithmic curves mapping the sensor sensitivity characteristics for the several substances. Indeed, TGS2602 sensitivity characteristic for ethanol shows a slope $\alpha_{TGS2602_{Eth}}$ equals to -0.416939 (just as for toluene, $\alpha_{TGS2602_{Tol}}$ equals to -0.564573), whereas for ammonia $\alpha_{TGS2602_{NH_3}}$ is -0.28658. Temperature and humidity values were constant at about, respectively, 23 °C and 70%

bon monoxide) pico-liters of ethanol (but in a quantity still lower than for TGS2602) were placed on the bottom of desiccator. It was not possible to involve the other substances, which TGS2620 is sensitive to, in the tests. The voltage outputs are shown in Fig.2.4. Because of a quantity of ethanol

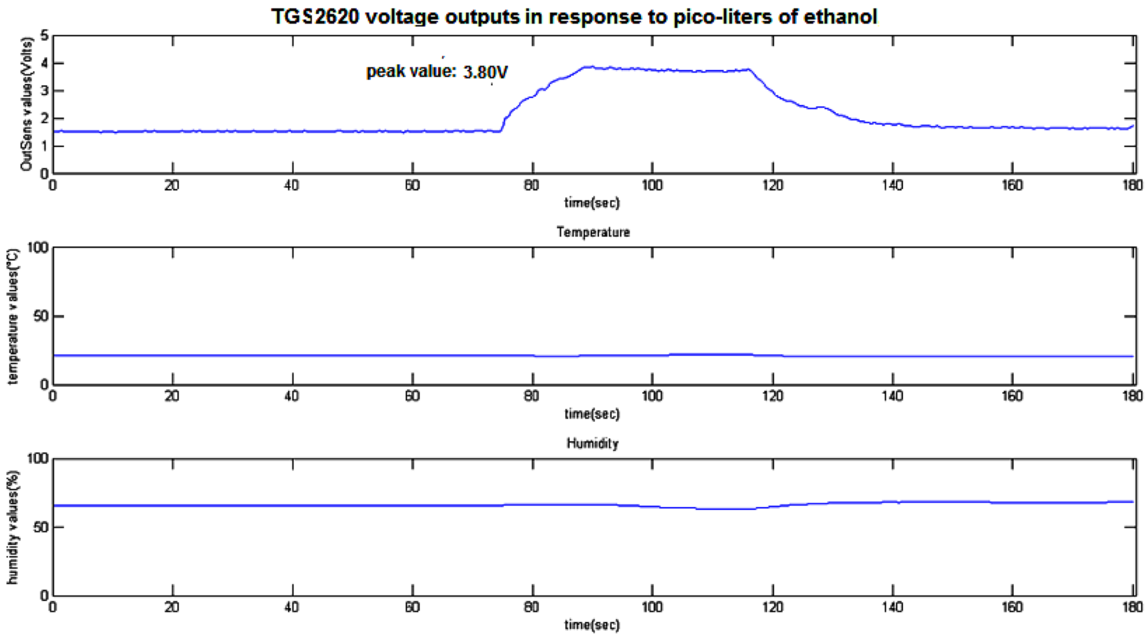

Figure 2.4: **TGS2620 outputs in response to ethanol concentration on the order of pico-liters.** Temperature and humidity values were constant at about, respectively, 23 °C and 70%

lower than for TGS2602, the TGS2620 output voltage rise is more slowly, although TGS2620 is more sensitive to ethanol than TGS2602 ($\alpha_{TGS2620_{Eth}}$ is -0.676428).

In both cases, as shown in figures, temperature and humidity values were constant at about, respectively, 23 °C and 70%.

2.2 Test on a population

A second phase of our trials was focused on the evaluation of device's performances when used to sense human exhaled gases.

2.2.1 Measuring protocol

First of all, a measuring protocol was draft basing on several aspects:

- First, the aim: what was the "Was Sniffer" thought to? The device has been thought to be integrated in the "Wize Mirror" in order to help the individuals not to make a "diagnosis", but to evaluate their wellness state, their life-sytle, focusing on cardio-metabolic risk.
  As a consequence, our breath analysis has been focused on those substances correlated to particular noxious habits, such as alcohol intake, cigarette smoke, metabolic stress induced by carbohydrates over-load.

- What about the target population? The atherosclerotic illness develops insidiously, and clinical manifestations often become evident in its advanced stages. Altogether, the correlated diseases and complications occur between 40 and 60 years of age.
  As a consequence, since it's talking about prevention, we considered a population between 20 and 50 years of age, containing individuals with different habits (smokers/non smokers, sporty/sedentary lifestyle, healthy/unhealthy diet, teetotal/social drinker).

- Methodological issues for sampling collection: substances in exhaled breath may be blood borne (systemic) and originate from the alveoli; others are also generated in the airways, and still other substances represent contaminants from ambient air. As a consequence, several factors may impact on the composition of breath samples: diluition and contamination of the sample(diluition with dead-space air, or contamination with exogenous compounds, included the ones originated in the oral cavity and produced by bacteria); sampling of single or multiple breaths; physiological parameters such as respiratory rate or cardiac output (the higher the ventilation flow, the lower the actual concentration of systemic compounds in exhaled breath because of diluition; the higher the cardiac output, the higher the supply of systemic volatile compounds).

In practice are often used three methods of sampling: "alveolar (end-tidal) sampling" (which corresponds to the plateau of the CO_2 curve, that is the maximum value of exhaled CO_2),"mixed expiratory air sampling" (which corresponds to a whole breath sample), "time-controlled sampling" (which corresponds to a part of exhaled air sampled after the start of expiration).

Controlled alveolar sampling, by means, for example, of expired CO_2 concentrations, is the method of choice if systemic volatile biomarkers are to be assessed, since only alveolar compounds are correlated to compounds in blood. The mixed expiratory air sampling, without controlled identification of the respiratory phases bears the risk of diluition with dead space air, but this method allows to collect also the compounds of exogenous origin. The latter method shows large variations of samples compositions because of wide variations of individual dead space volumes and breathing manouvres, then it is less used in clinical practice.

For our purposes, **mixed expiratory air sampling** method was chosen, since our interest was focused on endogenous biomarkers, but also to the compounds of exogenous origin (ethanol, for example).

Additionally, since the composition of single breaths may vary considerably from each other, because of different modes and depht of breathing, in order to have breath samples that were as reproducibles as possible, we prefered a sampling of **multiple breaths**.

The cardiac output was monitored and documented for each subject.

Basing on these considerations, the measuring protocol used was the following:

- **Population**: 20 subjects, of which 9 female and 11 male;
  age of subjects: 6 in RANGE1 [20-29 years]; 7 in RANGE2 [30-39 years];5 in RANGE 3 [40-49 years]; 2 in RANGE 4 [50-60 years];
  body type: 2 underweight; 14 normal; 4 overweight;

- **Alcohol intake: evaluate whether the device is able to discriminate between two different alcoholic grades**;
  - first, the subject (belonging to the population described above) makes 3 whole breaths in the Wize Sniffer; the data from the device are saved in a text file. The data relative to the subject (age, weight, habits)and to his heart rate (taken by means of a sphygmomanometer) are registered.
  - the same subject makes 3 whole breaths in the Wize Sniffer 15-30 min.[1] after the intake of 2 drinks of beer (alcohol grade: 5

[1]Most of the alcohol consumed is metabolized in the liver, but the small quantity that remains unmetabolized (about 2-3%) permits alcohol concentration to be measured in breath and urine. The liver can metabolize only a certain amount of alcohol per hour (an average individual metabolizes around 13ml per hour), regardless of the amount that has been consumed. The rate of alcohol metabolism depends, in part, on the amount of metabolizing enzymes in the liver, which varies among individuals. In general, after the consumption of one standard drink (a drink which contains about 14g of pure alcohol),

%)fasting; the data from the device are saved in a text file. The data relative to a blood alcohol concentration (taken by means of a simple blood alcohol tester) are registered.

- the same subject makes 3 whole breaths in the Wize Sniffer after 15-30 min. the intake of 2 drinks of wine (alcohol grade: 12 %);the data from the device are saved in a text file. The data relative to the blood alcohol concentration (taken by means of a simple blood alcohol tester) are registered.
- the test can be performed taking another subject and making him eat before alcohol intake[2].

- **Alcohol intake: evaluate whether the device is able to discriminate different amounts of the same drink**
  - first, the subject (belonging to the population described above) makes 3 whole breaths in the Wize Sniffer; the data from the device are saved in a text file. The data relative to the subject (age, weight, habits)and to his heart rate (taken by means of a sphygmomanometer)are registered.
  - the same subject makes 3 whole breaths in the Wize Sniffer 15-30 min. after the intake of 1 drink of beer(/wine) (alcohol grade: 5

the amount of alcohol in the drinker's blood (blood alcohol concentration, or BAC) peaks within 30 to 45 minutes.

[2]The presence of food and the type of food in the gastrointestinal tract when alcohol is consumed influence the alcohol absorption process itself. The rate at which alcohol is absorbed depends on how quickly the stomach empties its contents into the intestine. The higher the dietary fat content, the more time this emptying will require and the longer the process of absorption will take. Usually, subjects who drank alcohol after a meal that included fat, protein, and carbohydrates absorbed the alcohol about three times more slowly than when they consumed alcohol on an empty stomach.

%(/12%)) fasting; then, the test is repeated after the intake of a second drink of beer (/wine); the data from the device are saved in a text file. The data relative to the blood alcohol concentration (taken by means of a simple blood alcohol tester) are registered.

- the test can be performed taking another subject and making him eat before alcohol intake.

- **Alcohol intake: assess whether the device is able to follow the trend in time of the alcohol disposal**

  - first, the subject (belonging to the population described above) makes 3 whole breaths in the Wize Sniffer; the data from the device are saved in a text file. The data relative to the subject (age, weight, habits) and to his heart rate (taken by means of a sphygmomanometer) are registered.
  - the same subject makes 3 whole breaths in the Wize Sniffer each 15-30 min whithin 1 hour after the intake of 1 or 2 drink of beer(/wine) (alcohol grade: 5 % (/12%)) fasting; the data from the device are saved in a text file. The data relative to the blood alcohol concentration (taken by means of a simple blood alcohol tester) are registered.
  - the test can be performed taking another subject and making him eat before alcohol intake.

- **Smoking habit: assess whether the device is able to discriminate between non smoker's exhaled gas and smoker's exhaled gas**

  - two subjects (belonging to the population described above), one smoker and the other one non-smoker, make 3 whole breaths in the Wize Sniffer; the data from the device are saved in a text file. The data relative to the subjects (age, weight, habits) and to his heart rate (taken by means of a sphygmomanometer) are registered.

- **Smoking habit: evaluate device's output in response to a subject's exhaled breath after smoking**
  - a smoker subject (belonging to the population described above) makes 3 whole breaths in the Wize Sniffer; the data from the device are saved in a text file. The data relative to the subjects (age, weight, habits) and to his heart rate (taken by means of a sphygmomanometer) are registered;
  - the same subject makes 3 whole breaths in the Wize Sniffer after smoking a cigarette; the data from the device are saved in a text file.
- **Smoking and alcohol intake: evaluate device's output in response to a subject's exhaled breath after smoking and drinking alcoholic drink**
  - a smoker subject (belonging to the population described above)makes 3 whole breaths in the Wize Sniffer; the data from the device are saved in a text file. The data relative to the subjects (age, weight, habits) and to his heart rate (taken by means of a sphygmomanometer) are registered;
  - the same subject makes 3 whole breaths in the Wize Sniffer after smoking a cigarette and drinking 100g of beer (/wine); the data

from the device are saved in a text file.

- **Metabolism: evaluate device's output in response to:**
  - overweight subject's exhaled breath;
  - normal body type subject's exhaled breath;
  - subject's exhaled breath after exercise;

2.2.2 Data analysis

The test data, saved in text files, were analyzed by means of a programme implemented on Matlab. The pseudocode is the following:

This programme opens the file .txt where the sensors' voltage outputs has been saved, and reshapes them in a matrix containing 10 rows (everyone corresponding to each sensor: TGS2442, TGS2660, TGS821, TGS2602, TGS2444, TGS4161, Cosmed CO_2 sensor, Cosmed O_2 sensor, temperature values, humidity values). Then, creates vectors 1xn, each of which corresponding to a specific sensor. The baseline values are set to zero, and for each vector the maximum voltage value is saved in another vector.
Since the ones manufactured by Figaro are semiconductor gas sensors, which functioning is based on a variation of internal resistance (Rs) when they sense gas molecules, then this Rs had to be calculated[3], starting from the output voltage, according to the voltage divider equation present in the datasheets:

$$Rs = RL\frac{Vc - V_{out}}{V_{out}} \tag{2.1}$$

where RL is the load resistance on which the V_{out} is taken, and Vc is the sensor power supply. In addition, the ratio Rs/Ro (where Ro is the resis-

[3]except for TGS4161 which output interpretation is different

- ✓ Opening data text file and creating a matrix of values:
  - ❖ *fopen ('data.txt');*
  - ❖ re-shaping of the data the file in a matrix :
    mat[x rows, 10 columns (=number of data to read)]
- ✓ From matrix → to vectors (one for each sensor)
  - ❖ *SENSOR(i)=(mat(:,(i));*
    avg(i)=(sum (SENSOR(i)(1:9))/(length(SENSOR(i)(1:9)));
    SENSOR(i)= SENSOR(i)- avg(i); %set baseline values to zero
- ✓ For each "gas sensor vector", the maximum voltage value is saved in another vector V;
  - ❖ *V(i)=max(SENSOR(i));*
- ✓ From voltage value → to Rs → to Rs/Ro ratio ...
  - ❖ *Rs(i)=((Vc-V(i))/V(i))*Rl; %voltage divider equation*
    RATIO(i)=Rs(i)/R0(i);
- ✓ → to substances concentrations by means of "mysyst" function
  - ❖ *CONC(i)=(RATIO(i)/A_i_sub)^(1/alpha_i_sub);*
- ✓ Data plot

Figure 2.5: **Pseudocode of the prgramme implemented on matlab to analyze the data**

tance reference value and it is different for each sensor) had to be calculated, according to sensors' sensitivity characteristics which map the ratio Rs/Ro as a function of the substance concentration (see Chapter 1). In particular, these sensitivity characteristics derive from a power low such as:

$$\frac{Rs}{Ro_{TGSxxxx}} = A_{TGSxxxx_{sub}}[sub]^{\alpha_{TGSxxxx_{sub}}} \tag{2.2}$$

The constant parameters $A_{TGSxxxx_{sub}}$ and $\alpha_{TGSxxxx_{sub}}$ are different for each sensor. They has been calculated fitting the bilogarithimc curves representing the sensitivity characteristics. For this purpose, the software "Gnuplot" has been used (see Appendix A). Starting from the knowledge, for each Figaro sensor, of the ratio Rs/Ro, and of the constant parameters $A_{TGSxxxx_{sub}}$

and $\alpha_{TGSxxxx_{sub}}$, the function "mysyst", calculates the concentrations of the substances detected by TGS2442, TGS821, TGS2620, TGS2602, TGS2444, according to the following system of non-linear equations[4]:

$$\begin{cases} (\frac{Rs}{Ro})_{2442}=A_{2442_{\mathrm{CO}}}[\mathrm{CO}]^{\alpha_{2442_{\mathrm{CO}}}} \\ (\frac{Rs}{Ro})_{821}=A_{821_{\mathrm{H_2}}}[\mathrm{H_2}]^{\alpha_{821_{\mathrm{H_2}}}} \\ (\frac{Rs}{Ro})_{2444}=A_{2444_{\mathrm{NH_3}}}[\mathrm{NH_3}]^{\alpha_{2444_{\mathrm{NH_3}}}} \\ (\frac{Rs}{Ro})_{2620}=A_{2620_{Eth}}[Eth]^{\alpha_{2620_{Eth}}}+A_{2620_{\mathrm{H_2}}}[\mathrm{H_2}]^{\alpha_{2620_{\mathrm{H_2}}}}+A_{2620_{\mathrm{CO}}}[\mathrm{CO}]^{\alpha_{2620_{\mathrm{CO}}}} \\ (\frac{Rs}{Ro})_{2602}=A_{2602_{Eth}}[Eth]^{\alpha_{2602_{Eth}}}+A_{2602_{\mathrm{H_2}}}[\mathrm{H_2}]^{\alpha_{2602_{\mathrm{H_2}}}}+A_{2602_{\mathrm{H_2S}}}[\mathrm{H_2S}]^{\alpha_{2602_{\mathrm{H_2S}}}}+A_{2602_{\mathrm{NH_3}}}[\mathrm{NH_3}]^{\alpha_{2602_{\mathrm{NH_3}}}} \end{cases} \tag{2.3}$$

This system resulted from three hypothesis:

- for the "multi-sensing" sensors (TGS2620 and TGS2602), we made the hypotesis that the several contributions from the several substances add together linearly;
- TGS2620 is sensitive to methane and isobutane, too. Since the sensitivity range for methane and isobutane (50-5000ppm) is higher than the amounts of these substances present in our breath (less than 2 ppm), we made the hypotesis that their contributions were null; so:

$$A_{2620_{\mathrm{CH_4}}}[\mathrm{CH_4}]^{\alpha_{2620_{\mathrm{CH_4}}}} = 0 \tag{2.4}$$

$$A_{2620_{Isob}}[Isob]^{\alpha_{2620_{Isob}}} = 0 \tag{2.5}$$

- the same, for the toluene, which TGS2602 is sensitive to. TGS2602's sensitivity range for toluene is 1-10ppm, while in our breath this compound is present in lower amounts (less than 1 ppm); so:

$$A_{2602_{Tol}}[Tol]^{\alpha_{2602_{Tol}}} \tag{2.6}$$

[4]for convenience, the subscript "TGSxxxx" has been substituted with "Txxxx" only (for example, "TGS2442" becomes "2442")

The assessment of the CO_2 concentration, detected by TGS4161, results from an equation simpler than the previous; the datasheet of TGS4161 module (CDM4161A) suggests:

$$[CO_2] = 1000V_{out} \tag{2.7}$$

For COSMED sensors, CO_2 and O_2 concentrations can be assessed by means of the following table:

CO_2	O_2
0.02V +/-0.01V @ 0.03%	0 to 4V+/-0.01V @20.93%
1.75V +/-0.01V @ 2.00%	
3.5V +/-0.01V @ 5.00%	

Finally, the program implemented on Matlab allows to plot the data, mapping, for each sensor, the voltage output as a function of time (naturally, according to the test period).
As well known, there will be an increase in voltage if the sensor senses the gas which is sensitive to; the maximum voltage value will be related to the amount of substance that the sensor sensed.

2.2.3 Results and discussion

The most meaningful results are reported in this section, so as the "Wize Sniffer 1.0" performances are discussed, in order to make it ready to be integrated in the "Wize Mirror".
The tests were conducted, as per protocol, on 12 subjects (of which 6 female and 6 male); 6 of them were in age range 1 [20-29 years]; 2 of them were in the range age 2 [30-39 years]; 3 of them in range age 3 [40-49 years]; 1 of them in range age 4 [50-60 years]; in addition, 2 subjects were underweight,

7 had normal body type, 3 were overweight.
In detail:

1.Asymptomatic subject: typical curves

The test was conducted on a non-smoker, teetotal, famale subject belonging to the first age range, having normal body type, practising sport 2 times a week. The subject's heart rate was taken by means of a sphygmomanometer (CS 410, Pic Indolor), then the subject made 3 wholes breath into the device by means of the corrugated tube. The results relative to breath gases concentrations are shown in table 2.1, while the curves mapping the gas sensors' outputs are shown in Figure 2.6. In the table's first row, the baseline concentrations are shown for each substance. It should be noted that these values are purely approximate: the one of "breath analysis" is a very innovative field, and, as a consequence, the gases' concentration range, that might be attributed to a healthy (or asymptomatic) subject's exhaled breath, are not still standardized. For example, CO concentration may vary from 0.6ppm to 4.9ppm, for a healthy subject; these values may rise in a moderate smoker until 10-20ppm; in a strong smoker the values may reach 30ppm. We reported the concentration values typical of an asymptomatic subject.
In Figure 2.6 the typical trend of "exhaled breath curves" can be evaluated: few seconds after the sensors sense exhaled gas particles, the sensors' internal resistance varies, resulting in a voltage output rise, until the plateau curve. When the store chamber is purged, the voltage values come back (more or less slowly) to zero.

2.Alcohol intake: evaluate whether the device is able to discriminate between two different alcoholic grades

The tests were conducted on a non-smoker, social drinker, male subject belonging to the second age range, having normal body type, practising sport

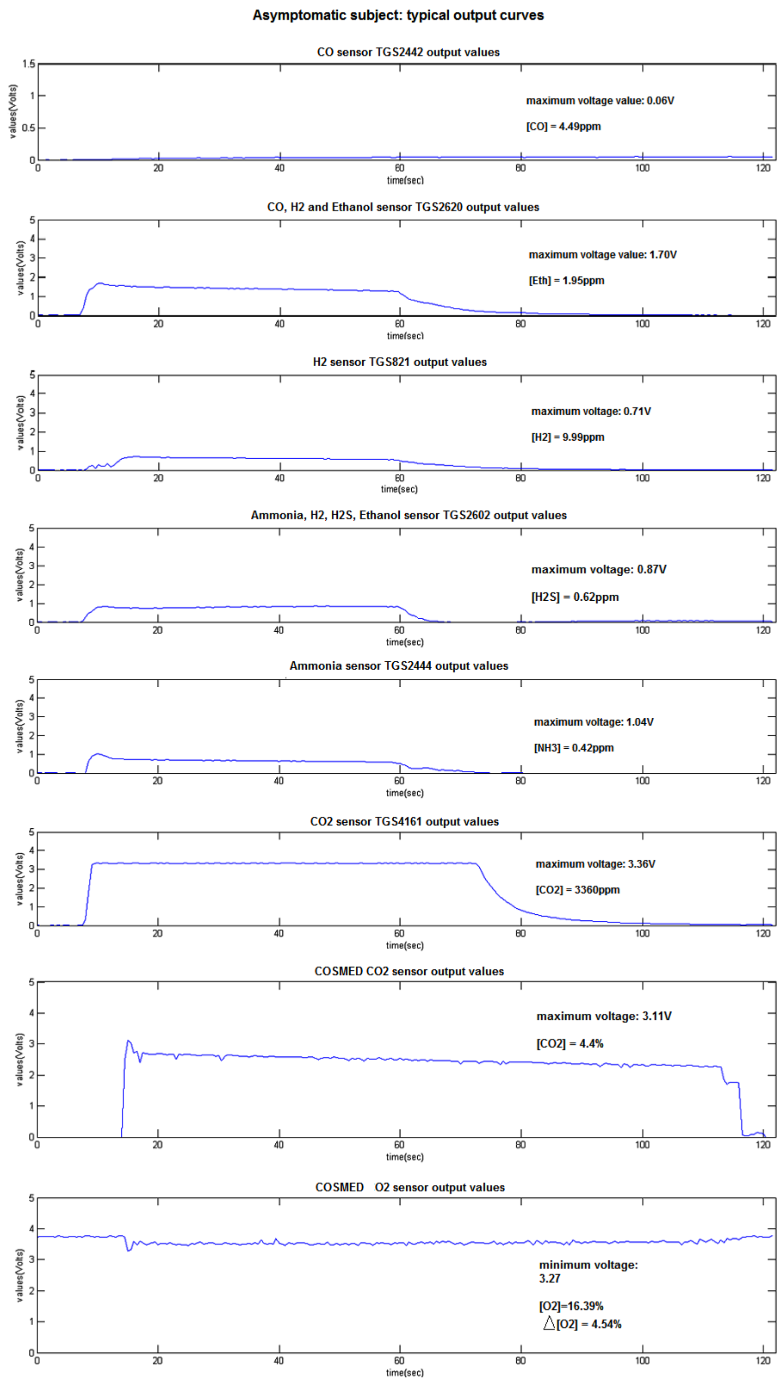

Figure 2.6: **Typical output curves in response to an asymptomatic subject's exhaled breath.** Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

Table 2.1: **Asymptomatic subject**

	HR(bpm)	[CO]ppm	$[H_2]$ppm	[Eth]ppm	$[H_2S]$ppm	$[NH_3]$ppm	$[CO_2]_F$ppm	$[CO_2]_C$	$[O_2]$
BL	60-70	0.6-4.9	0.3-34.1	0-3.9	0-1.3	0-1.3	4000	4%	14%
S_1	70	4.49	9.99	1.95	0.62	0.42	3380	4.4%	16.39%

1 time a week on average. The subject's heart rate was taken by means of a sphygmomanometer (CS 410, Pic Indolor).
First, the subject made 3 wholes breath into the device by means of the corrugated tube. Then, the test has been repeated 20 min after the subject drank 2 drinks (each one equals to 100ml) of beer (alcoholic grade 5%) fasting. The same trial has been repeated the day after, but making the same subject drink wine (alcoholic grade 12%) in same amount, fasting.
The results relative to breath gases concentrations are shown in table 2.2, while the curves mapping the gas sensors' outputs are shown in Figure 2.7.

Table 2.2: **Alcohol intake: discrimination between two different alcoholic grades**.Remember that the baseline value for exahled ethanol is 0-3.9ppm

HR	[Eth] without alcohol intake	[Eth] 20min. after 2x100ml of beer	[Eth] 20min after 2x100ml of wine
60-70bpm	3.86ppm	31.98ppm	71.32ppm

The results relative to ethanol concentrations, after 200ml of wine intake and then 200ml of beer intake, was validate by means of an alcohol tester (digital ethylometer Alcolino 126). The table 2.3 shows the comparison between these results. The BrAC value can be estimated dividing the BAC value for a constant parameter equals to 2100-2300. This is due to the constant ratio between blood ethanol and exhaled ethanol: 80mg of ethanol in 100ml of blood produce 0.035 mg/100ml of ethanol in exhaled breath.

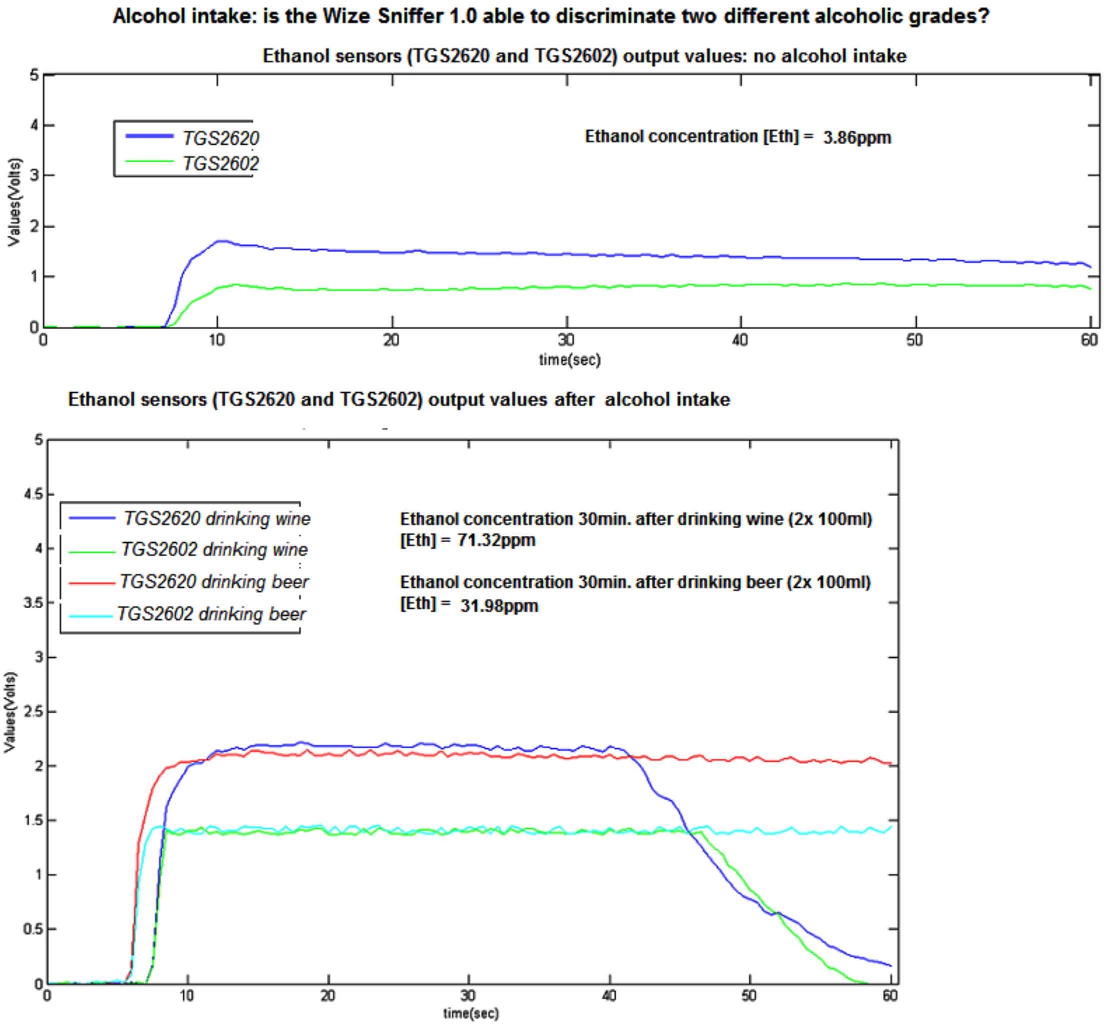

Figure 2.7: **Alcohol intake: discrimination between two different alcoholic grades. It should be noted that the sensor TGS2602 is able to discriminate between "asymptomatic subject" and "subject after drinking alcohol", but is not able to discriminate between different alcoholic grades. This may be due to the TGS2602 low sensitivity for ethanol: $\alpha_{TGS2602_{Eth}}$ is about -0.4 against $\alpha_{TGS2620_{Eth}}$ which is -0.6. Indeed, TGS2620 is more sensitive and discriminates well the two alcoholic grades.**Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

Table 2.3: **Comparison between Wize Sniffer 1.0 output ethanol concentration and Alcolino output Blood Alcohol Concentration (BAC). From BAC value, the BrAC one can be estimated.**

	Wize Sniffer [Eth]	Alcolino 126 BAC	Alcolino 126 BrAC
after 200ml of beer	31.98ppm	150mg/l+/-10%	39.66ppm+/-10%
after 200ml of wne	71.32ppm	220mg/l+/-10%	57.77ppm+/-10%

.

We can affirm that the device is able to discriminate the different alcoholic grades and returns in output breath ethanol concentrations very close to the ones output from alcohol breath tester.

3.Alcohol intake: evaluate whether the device is able to discriminate different amounts of the same drink The tests were conducted on a non-smoker, social drinker, male subject belonging to the first age range, having normal body type, not practising sport. The subject's heart rate (73bpm) was taken by means of a sphygmomanometer (CS 410, Pic Indolor).

First, the subject made 3 wholes breath into the device. Then, the test has been repeated 20 min after the subject drank the first drinks (100ml of wine, alcoholic grade 12%) fasting. The same trial has been repeated 20 min after the second drink of wine (drunk 45min after the first one).

Then, two test were repeated (whit a gap of 20min from each other and from the last one), in order to **evaluate if the device was able to follow the follow the trend in time of the alcohol disposal**.

The results relative to breath gases concentrations are shown in table 2.4, while the curves mapping the gas sensors' outputs are shown in Figure 2.8.

As shown in table 2.4 and in Figure 2.8, the ethanol sensor TGS2620 allows the device to give an output very close to the one output from alcohol tester. The voltage outputs showed an increase when the second drink was

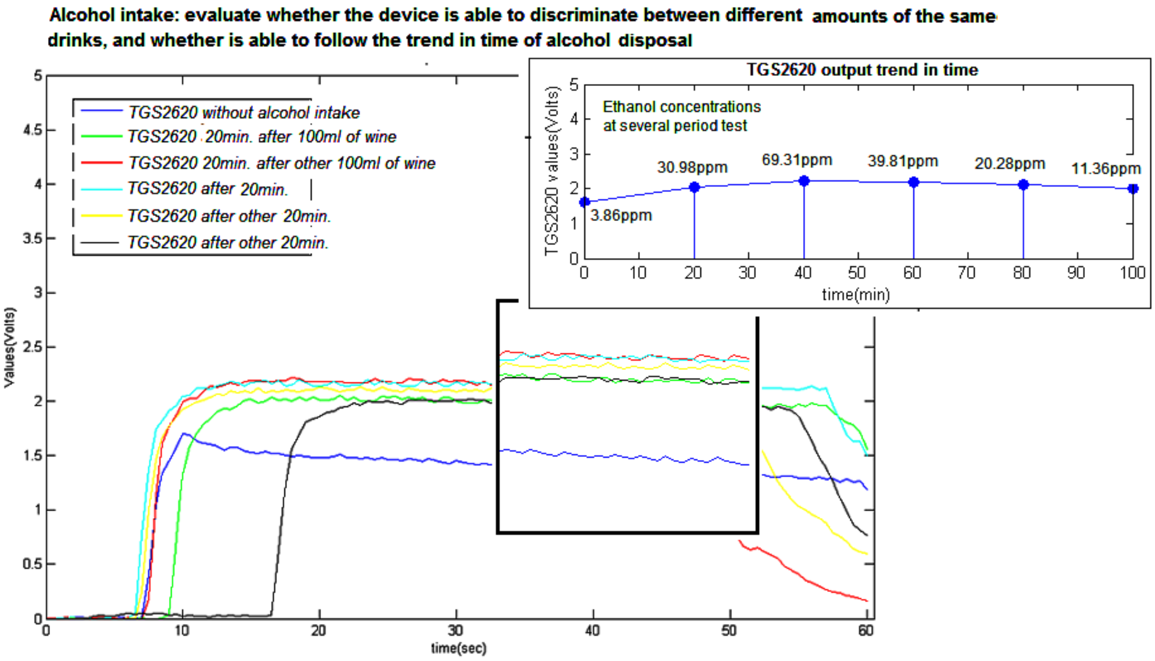

Figure 2.8: **Alcohol intake: the curves show the sensor TGS2620 output in response to different amount of wine drink. Then, three test has been repeated in order to evaluate if the device was able to follow the trend of alcohol disposal.**Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

Table 2.4: **Alcohol intake: different amount of the same drink (same alcoholic grade)and alcohol disposal-Wize Sniffer 1.0 output ethanol concentration and Alcolino output Blood Alcohol Concentration (BAC). From BAC value, the BrAC one can be estimated.**

	Wize Sniffer [Eth]	Alcolino 126 BAC	Alcolino 126 BrAC
without alcohol intake	3.86ppm	0.00mg/l	
20min. after 1st 100ml of wine	30.98ppm	120mg/l+/-10%	33.66ppm+/-10%
20min. after 2nd 100ml of wine	69.31ppm	280mg/l+/-10%	73.88ppm+/-10%
after 20min	39.81ppm	160mg/l+/-10%	42.27ppm+/-10%
after 40min	20.28ppm	110mg/l+/-10%	29.05ppm+/-10%
after 60min	11.36ppm	50mg/l+/-10%	13.22ppm+/-10%

swallowed, and a decrease when alcohol disposal began.
Then, we can affirm that the Wize Sniffer 1.0 is able to follow the trend in time not only of alcohol intake but also of alcohol disposal.
4.Alcohol intake: evaluate whether the device is able to distinguish between alcohol intake fasting and alcohol intake after eating.
As well known, an individual swallowing alcohol fasting will metabolize alcohol more slowly as opposed to an individual swallowing alcohol after eating. Then, a test was conducted on a non-smoker, social drinker, male subject belonging to the third age range, having normal body type, not practising sport. The subject's heart rate (69bpm) was taken by means of a sphygmomanometer (CS 410, Pic Indolor).
First, the subject made 3 wholes breath into the device. Then, the test has been repeated 20 min after the intake of 2x100ml of beer (alcoholic grade 5%) fasting. The same trial has been repeated the day after, but making the subject eat a sandwich before drinking.
The results relative to breath gases concentrations are shown in table 2.5, while the curves mapping the gas sensors' outputs are shown in Figure 2.9.

In this case, the numerical values relative to ethanol concentrations output

Table 2.5: **Discrimination between alcohol intake fasting and alcohol intake after eating-Wize Sniffer 1.0 output ethanol concentration and Alcolino output Blood Alcohol Concentration (BAC). From BAC value, the BrAC one can be estimated.**

	Wize Sniffer [Eth]	Alcolino 126 BAC	Alcolino 126 BrAC
200ml of beer fasting	40.42ppm	0.13mg/l+/-10%	34.26ppm+/-10%
200ml of beer after eating	20.18	0.09mg/l+/-10%	23.77ppm+/-10%

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from our device was not as close to the ones output from Alcolino as in the previous case. Nevertheless, the Wize Sniffer 1.0 was able to discriminate well the two different test conditions on a quality level.

5.Smoking habit: to evaluate whether the device is able to discriminate between moderate smoker subject and non-smoker subject's exhaled gas; evaluate also the device's output in response to a subject's exhaled breath after smoking and after smoking and drinking. The tests were conducted on a non-smoker female subject belonging to the first age range, having normal body type, practising sport 2 time a week on average, and on a moderate smoker female subject,belonging to the first age range, having overweight body type and not practising sport. The subjects's heart rate was taken by means of a sphygmomanometer (CS 410, Pic Indolor).

First, the non smoker subject made 3 wholes breath into the device by means of the corrugated tube. Then, the test has been repeated on the smoker subject. Then, another two tests were conducted: respectively, after the smoker subject smoked a cigarette, and after the same subject smoked and drank 100ml of beer.

The results relative to breath gases concentrations are shown in table 2.6,

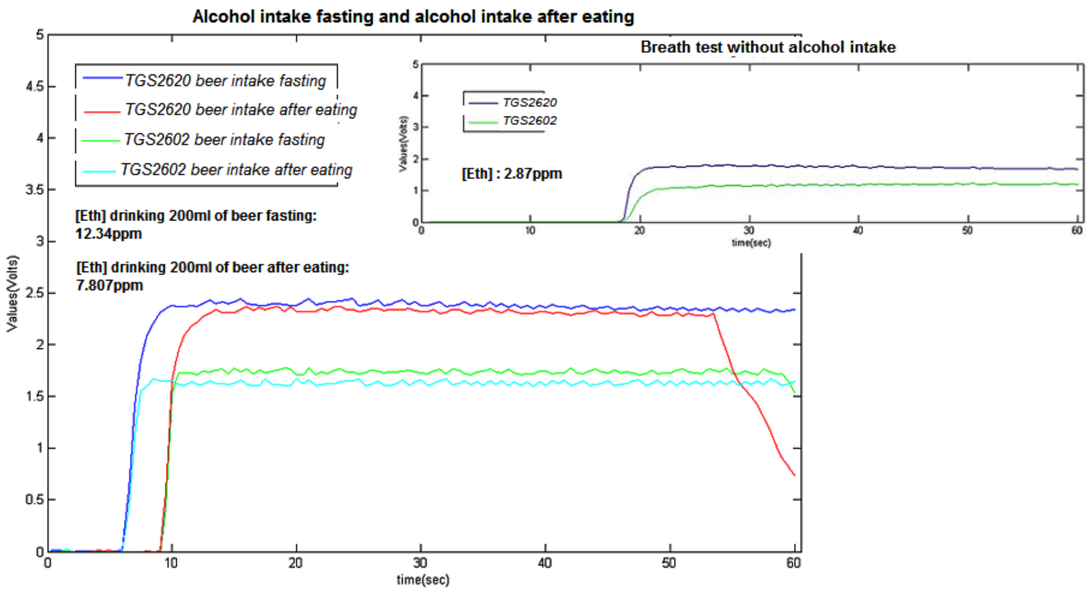

Figure 2.9: **Alcohol intake: the curves show the sensors TGS2620 and TGS2602 output in response to breath test performanced on a subject drinking 200ml of beer fasting and drinking 200ml of beer after eating a sandwich.** The difference between these two ethanol sensor in terms of sensitivity is evident($\alpha_{TGS2602_{Eth}}$ = 0.4 against $\alpha_{TGS2620_{Eth}}$ = 0.6). Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

while the curves mapping the gas sensors' outputs are shown in Figure 2.10 and Figure 2.11.

Several considerations derived from these trials:

- the device was able to discriminate between a non smoker subject and a moderate smoker subject; indeed, the values corresponding to carbon monoxide concentration, just like the ones corresponding to carbon dioxide, were higher in a smoker individual than in a non-smoker; as a consequence, the oxygen concentrations followed the opposite trend, coherently to the fact that carbon monoxide in blood takes the place

Table 2.6: **Smoking habit: discrimination between non smoker subject and moderate smoker subject; effects of smoking on exhaled gas**

	HR	[CO]	[O_2]	[CO_{2F}]	[CO_{2C}]	[H_2]	[Eth]	[NH_3]
non-sm. subj	78bpm	4.49ppm	18.33 %	3320ppm	3.77%	3.49ppm	1.24ppm	0.41ppm
sm. subj	79bpm	9.19ppm	17.4%	3370	3.95%	1.39ppm	1.86ppm	0.26ppm
sm. subj after smoking	77bpm	20.7ppm	16.84 %	3398ppm	4.14%	3.462ppm	2.007ppm	0.46ppm
sm. subj after smoking and drinking	77bpm	30.04ppm	15.90 %	3368ppm	4.4%	2.47ppm	12.7ppm	0.89ppm

of oxygen, forming a more stable bond with haemoglobin;

- after smoking, both the times, the device detected higher values not only of carbon monoxide but also of ammonia, coherently to the presence of ammonia in cigarette smoke;
- the ethanol concentrations (12.7ppm) after alcohol intake (100ml of beer- alcoholic grade= 5%) were very close to the ones detected by means of alcohol tester (which returned an output of 0.06g/L for BAC, corresponding to 15.87ppm for BrAC);
- a limit for the detection of carbon monoxide may be the very low TGS2442 response time; although having good performance, the sensor was too slow not only in response, but also while coming back to its baseline value;
- Cosmed sensor for carbon dioxide had a performance better than TGS4161 (Figaro sensor for carbondioxide), which saturated too quickly. This may be due to Cosmed CO_2 sensor detection range which is wider than

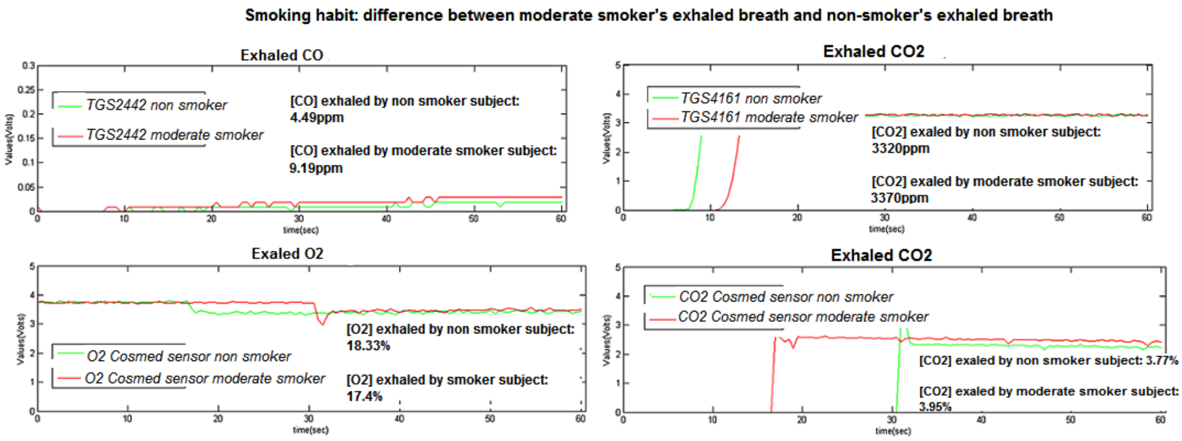

Figure 2.10: **Smoking habit: discrimination between non smoker's exhaled gas and moderate smoker's exhaled gas.** Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

the one of TGS4161. As a consequence, (as shown in Fig. 2.10), the first one was able to discriminate between smoker's exhaled CO_2 and non smoker's exhaled CO_2, much more than the second;

- an interesting aspect regarding the detection of exhaled hydrogen, was that exhaled hydrogen showed an increase after smoking, and a decrease after alcohol intake.

Regarding this last point, a change in exhaled hydrogen concentrations occurred also when the test 3) was conducted: there was a decrease in concentration values when the subject drank alcohol, and a return to initial values in the following 20-40 minutes. This is shown in Figure 2.12.
This event is in line with the bibliography: in the study conducted by D.G. Thompson et al.,[3], it was found that exhaled hydrogen showed a decrease in value after than the individuals made use of mouthwash. As described in the previous chapters, exhaled hydrogen derives from carbohydrate fermentation by anaerobic bacteria. This fermentation occurs not only in the colon, which

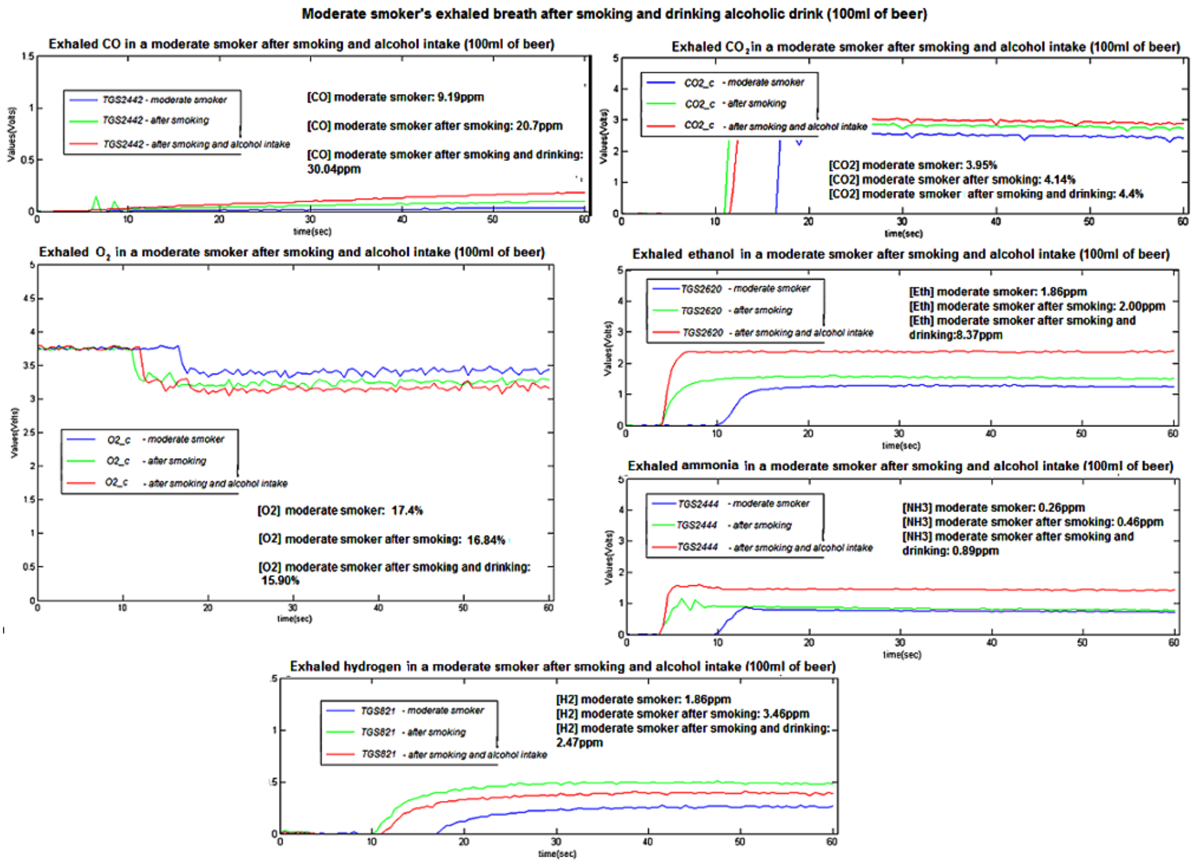

Figure 2.11: **Smoking habit: moderate smoker's exhaled breath after smoking and drinking alcohol.** Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

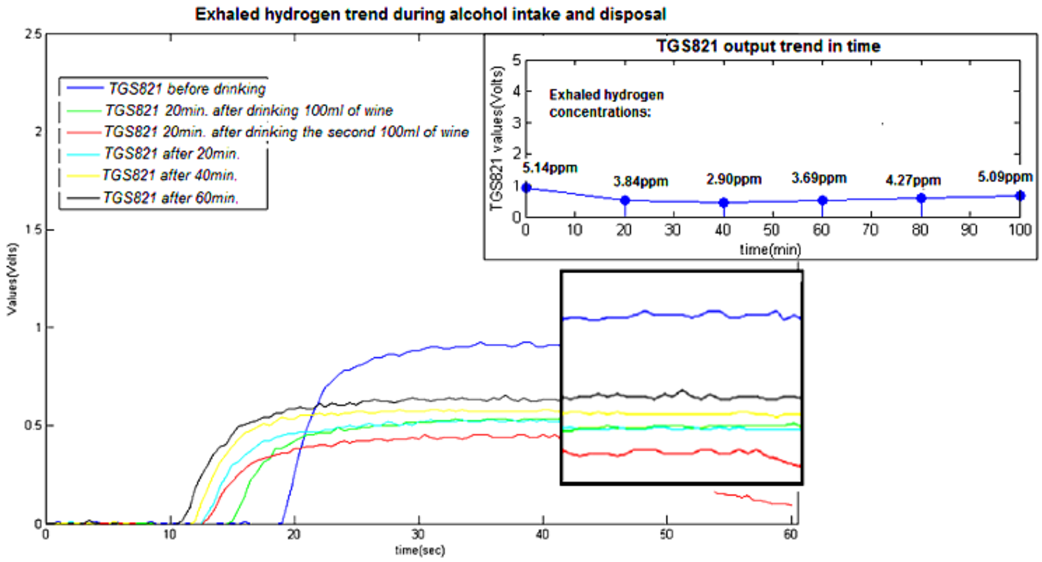

Figure 2.12: **The change in exhaled hydrogen concentrations can be seen clearly in the plot mapping the trend in time.** Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

is the normal human gastrointestinal tract that harbours anaerobic bacteria, but also in in the mouth and pharynx, which are colonised by anaerobes capable of carbohydrate fermentation. After making use of a mouthwash, containing alcohol and chlorhexidine (which functions as a broad-spectrum disinfectant against bacteria), the contribute of oral-origin exhaled hydrogen disappears.

In our case, the individual swallowed an alcoholic drink, so we can hypotesize that it had the same effect of mouthwash on subject's exhaled hydrogen.

6.Metabolism: evaluate device's output in response to:

- **overweight subject's exhaled breath;**
- **normal body type subject's exhaled breath;**

- **subject's exhaled breath after exercise;**

The tests were conducted on a normal body type male individual belonging to the second age range, and on an overweight female subject belonging to the first age range. The subjects's heart rate was taken by means of a sphygmomanometer (CS 410, Pic Indolor).
First, the subjects made 3 wholes breath into the device by means of the corrugated tube. Then, the test has been repeated on the overweight subject, after making her a running (upside-down the upstairs) for 5 minutes.
The results relative to breath gases concentrations are shown in table 2.7, while the curves mapping the gas sensors' outputs are shown in Figure 2.13.
As discussed in previous chapters, endogenous ethanol is originated from

Table 2.7: **Metabolism: device output concentration relative to exhaled ethanol and hydrogen- comparison between normalbody type subject (SUBJ1) and overweight subject (SUBJ2)**

	HR	[Eth]	[H_2]
SUBJ 1	57bpm	1.41ppm	5.49ppm
SUBJ 2	93bpm	3.04ppm	16.77ppm
SUBJ 2 after running	140bpm	2.07	6.173

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microbial fermentation of carbohydrates in the gastro-intestinal tract. Then, higher values of exhaled ethanol can be found,as in table 2.7, in individuals suffering overweight problems. The same for hydrogen values.
On the contrast, after running, there is a decrease in value for exhaled hydrogen. Again, in the study conducted by D.G. Thompson et al.,[3], it was found that exhaled hydrogen showed a decrease in value after excercise. The higher ventilation rate, because of exercise, reduces hydrogen concentration in the lungs.
As a consequence, also in our trial there was a decrease in exhaled hydrogen

"washed out" from the lungs by higher ventilation rate casused by running.

Naturally, there was a rise also in exhaled carbon dioxide values: 4.28% before running versus 5.39% after running.

2.2.4 Validation of results

From the tests, we gathered that the device is able to provide reliable outputs on a quality level.
In order to evaluate whether also its quantitative outputs were in line with bibliography, we considered worthwile to validate our results comparing them with the ones output by **Gas Chromatography** and the ones, relative to carbon monoxide, ouputs from **piCO+ Smokerlyzer**.

Validations of results by means of gas chromatography

The tests were conducted in collaboration with Dr. Maria Giovanna Trivella and her Breath Analysis research group of Institute of Clinical Physiology (I.F.C.) of C.N.R..
Unfortunately, we was able to validate only the results relative to exhaled ethanol detection: the gas chromatograph to our disposal could detect molecules with a molecular weight higher than 40g/mol. As a consequence, the other light analytes were not detected.
Then, our attention was focused on the comparison between exhaled ethanol concetrations output from Wize Sniffer, and the ones analysed by means of gas chromatograph.
Here, an example of the tests conducted is discussed.
Three subjects were taken from the population described previously: a fe-

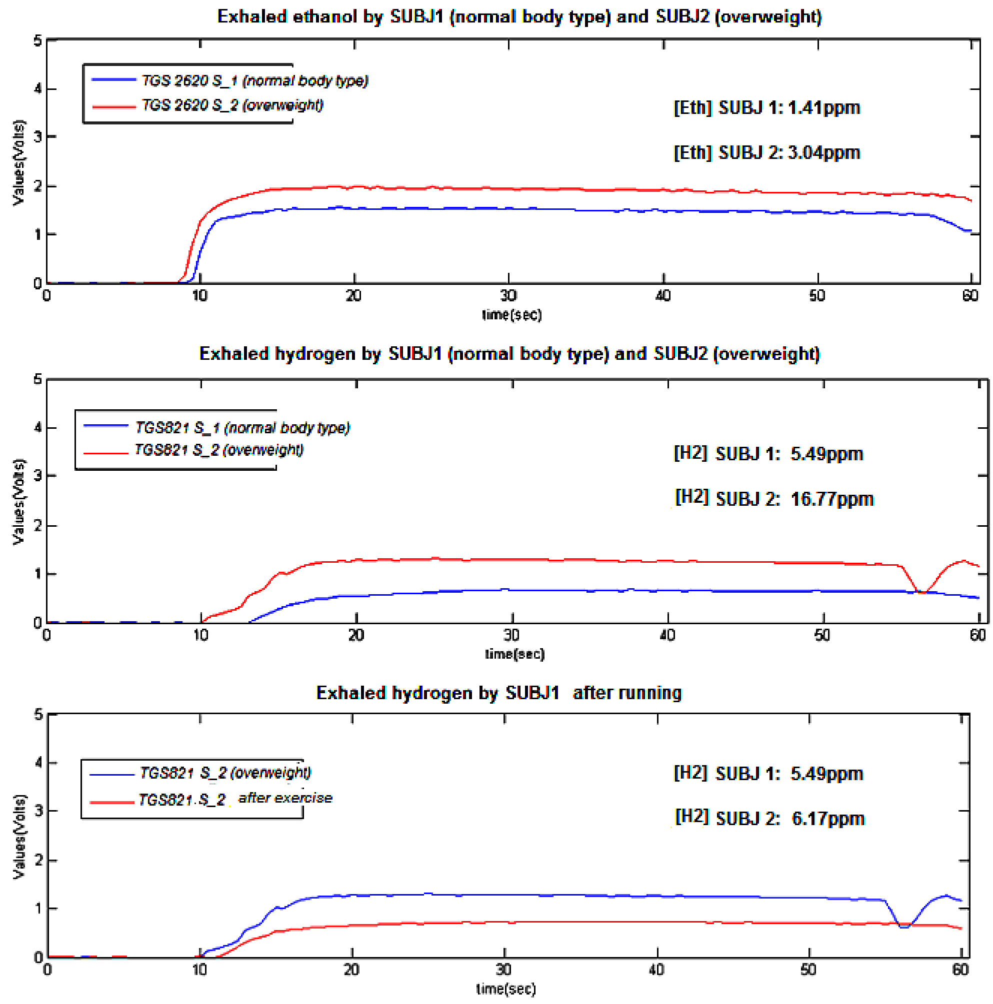

Figure 2.13: **Endogenous ethanol and hydrogen exhaled by normal body type subject (SUBJ1) and overweight individual (SUBJ2). The last plot maps SUBJ2 exhaled hydrogen concentration before and after running.** Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

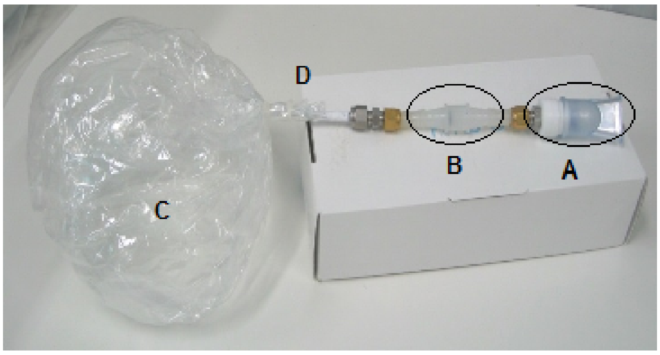

Figure 2.14: **The breath sampling bag consists of a disposable mouthpiece(A), a non-return valve (B), a Nalophan bag (C) and a Teflon tube (D). The Teflon tube allows the connection of the bag to the adsorption tube for transferring the breath sample. Each subject was asked to calmly fill the disposable bag with three deep breaths.**

male subject belonging to the first age range(SUBJ1) and quasi-teetotal, a male subject belonging to the first age range (SUBJ2), social drinker, and a female subject belonging to the second age range (SUBJ3), social drinker. All of them were normal body type.

The three subject, one by one, first made three whole breaths in the Wize Sniffer, and then 3 whole breaths in the disposable bag (see Fig.2.14) which is usually used in gas chromatography to sampling the exhaled breath.

Then, it has been made them swallow 100ml of alcoholic drink, whith different alcoholic grades. In particular, SUBJ1 drank 80ml of beer (alcoholic grade: 5%), SUBJ2 drank 80ml of prosecco (alcoholic grade 11%), SUBJ3 drank 80ml of wine (alcoholic grade: 13 %). After 20min. it was asked them to make three whole breaths in the Wize Sniffer, and another three in the disposable bags for gas chromatography.

Naturally, the Wize Sniffer returned a real-time output, whereas the gas cromatography required about about 1 hour for 1 sample.

Gas Chromatography results

The results relative to exhaled ethanol concentrations and deriving from gas chromatography are shown in table2.8. The ethanol concentrations relative to the first test (that is, before alcohol intake), were under LOD (limit of detection).

The difference between alcoholic grade was underlined by SUBJ2 and SUBJ3

Table 2.8: **Gas Cromatography results**. In the first column, the areas under peaks are shown; in the second one, the exhaled ethanol concentration (in ppmv) are reported. The gas chromatograph used was the one manufactured by Thermo Electron Corporation, model Finnigan TraceGC Ultra.

	Ethanol Area	Ethanol concentration (ppmv)
SUBJ 1-PRE	$< LOD$	$< LOD$
SUBJ 1-POST beer	4,44E+07	46,8
SUBJ 2-PRE	$< LOD$	$< LOD$
SUBJ 2-POST prosecco	3,46E+07	36,5
SUBJ 3-PRE	$< LOD$	$< LOD$
SUBJ 3-POST wine	4,28E+07	45,1

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exhaled ethanol. Neverthless, this comparison should not be made because alchol disposal varies between individuals and depends on several factor, among which sex, age, body mass, body fat, alcohol intake habit. Indeed, this was underlined by SUBJ1, quasi-teetotal, that is, little used to alcohol intake, who drank beer (which has an alcoholic grade lower than prosecco or wine) and exhaled more ethanol than SUB1 and SUBJ2.
The chromatograms mapping subjects' exhaled substances are shown in Fig.2.15.

Wize Sniffer results

The results from Wize Sniffer 1.0. relative to exhaled ethanol concentrations are reported in table2.9 and shown in Fig.2.16.

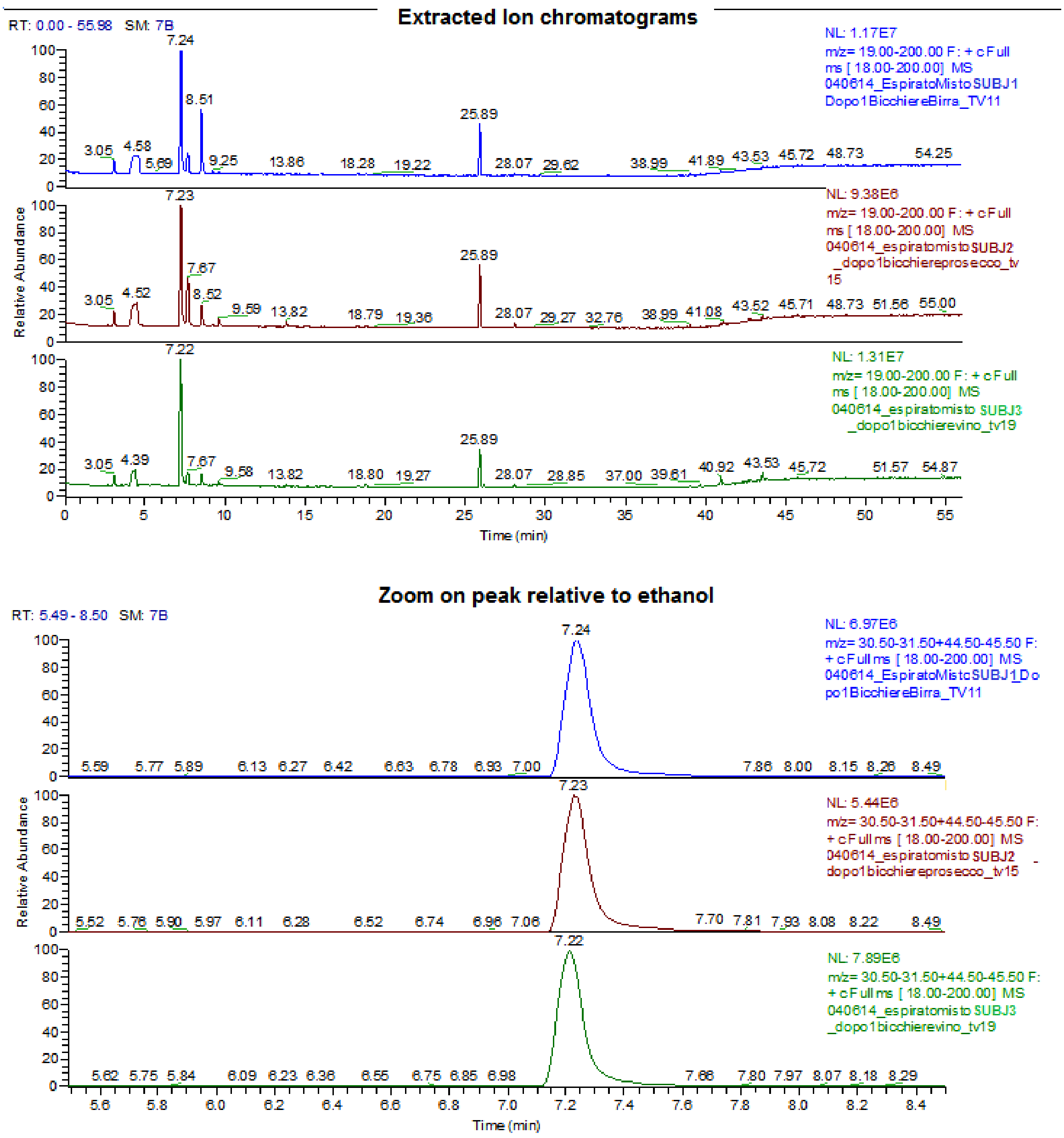

Figure 2.15: **The chromatograms relative to three subjects' exhaled substances. The peak, at min.7.22 is the one relative to ethanol (zoomed in the plots at the bottom).**

The device was able to return reliable results on a quality level, but, as we expected, there was not an overlapping with the nummerical results output from gas chromatography. Neverthless, this is understandable, considering the different resolutions of the two techniques.

In general, we can affirm that the device returned reliable outputs within

Table 2.9: **Wize Sniffer results**.

	Ethanol concentration (ppmv)
SUBJ 1-PRE	1.32ppm
SUBJ 1-POST beer	17.63ppm
SUBJ 2-PRE	4.08ppm
SUBJ 2-POST prosecco	10.17ppm
SUBJ 3-PRE	5.75
SUBJ 3-POST wine	25.27ppm

the several test conditions, on a quality level. Regarding numerical results, that is, breath analytes concentrations, further efforts should be made in order to improve

Validations of results by means of piCO+ Smokerlyzer

piCO+ Smokerlyzer (shown in Fig.2.17) is a device manufactured by Bedfont and commercialized by COSMED s.r.l..

It is a breath carbon monoxide monitor intended for multi-patient use by healthcare professionals in smoking cessation programmes, research and as an indicator of carbon monoxide poisoning in a healthcare environment. Breath carbon monoxide is measured in ppm; also blood carboxyhemoglobin is calculated in percentages (%COHb)[5]. Three subjects were taken from the

[5] CO competes successfully with oxygen in the bloodstream to form COHb. This starves thebody tissues of oxygen vital to repair, regeneration and general living. CO can remain in the blood stream for up to 24hours, depending on a range of factors including physical

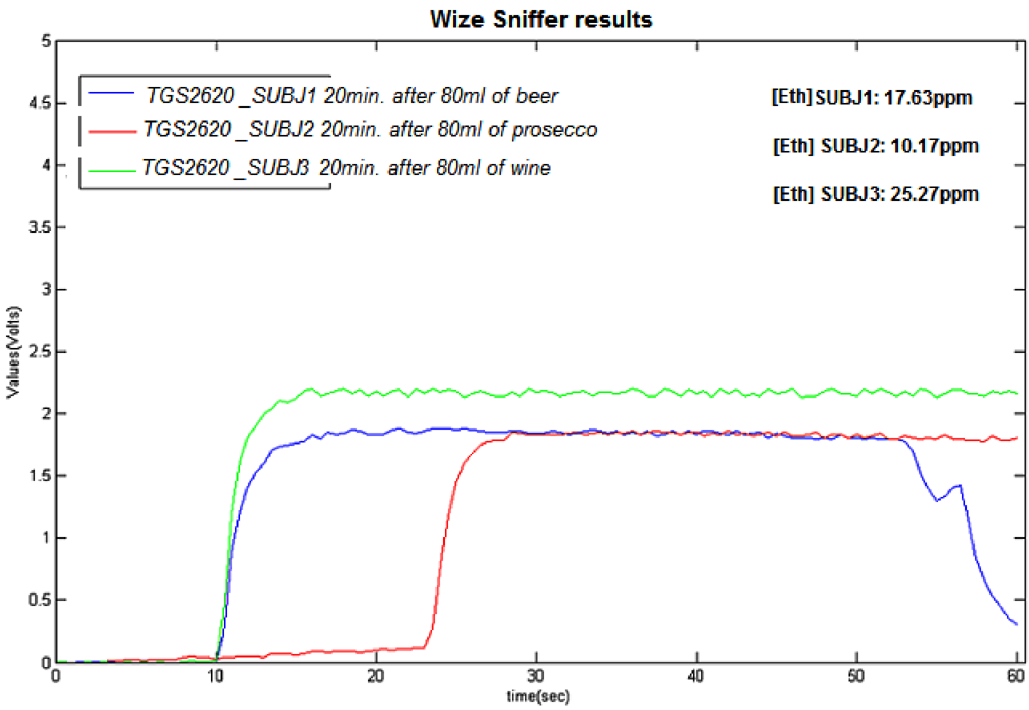

Figure 2.16: **Wize Sniffer results relative to three subjects exhaled ethanol after alcohol intake. SUBJ1 drank 80ml of beer, SUBJ2 80ml of prosecco, SUBJ3 80ml of wine.** Temperature and humidity values have ranged between 23-25 °C and 50%-60% respectively.

Figure 2.17: **piCO+ Smokerlyzer is a device manufactured by Bedfont and commercialized by COSMED s.r.l.**

population described previously: a male subject belonging to the second age range (SUBJ1), light smoker, a female subject belonging to the second age range (SUBJ2), moderate smoker, a female subject belonging to the first age range(SUBJ3),heavy smoker. All of them were normal body type.
The three subject, one by one, first made three whole breaths in the Wize Sniffer, and then 1 whole breaths in the piCO+ Smokerlyzer.
The tests were repeated after the subjects smoked a cigarette.
The results are shown in table 2.10. The device was able to return reliable results both on a quality level and numerically.
In this case, an overlapping could be expected, considering that both the device (piCO+ Smokerlyzer and Wize Sniffer 1.0.) base their function on a gas sensor.

activity, gender and inhalation intensity. The half life is about 5 hours.

Table 2.10: **Smoking habit: validations of results by means of piCO+ Smokerlyzer**.

	smoking habits	PRE-WS	PRE-piCO+	POST-WS	POST-piCO+
SUBJ 1	1-2 cig./day	4.3ppm	3ppm	11.42	8ppm
SUBJ 2	4-5 cig./day	5.9ppm	7ppm	13.01ppm	11ppm
SUBJ 3	20 cig./day	9.12ppm	11ppm	20.7ppm	17ppm

Conclusions and future developments

In this work we have presented the hardware and software design of a first prototype of the so called "Wize Sniffer 1.0", a portable device for breath analysis operating on an effective VOCs, provided by the European SEMEOTICONS Project (www.semeoticons.eu) ([2]).
Our aim has been to see that the device returned a sort of "breathprint" for every individual (similar to a fingerprint), that could provide useful informations about his or her state of health and, in case, give a feedback about alcohol intake, smoking habit, improper lifestyle in general. Naturally, this "breathprint" is based on the detection of a limited number of substances, suggested by physicians and related to cardio-metabolic risk prevention.
As a consequence, the detected exhaled analytes were: carbon monoxide (the major component present in tobacco fumes), hydrogen (resulting from carbohydrate fermentation by anaerobic bacteria), ethanol (endogenous, originated from microbial fermentation of carbohydrates in the gastro-intestinal tract, and exogenous, deriving from alcoholic drink), ammonia (another component present in tobacco fumes), carbon dioxide (which production can be considered as a measure of metabolism), oxygen.
In this report was described:

- the design of the hardware platform, including the Arduino Ethernet board, the circuit for the connection between Arduino board and an array of commercial sensors;
- the communication protocol between sensors and the notebook(laptop) both via serial cable or wireless;
- the functionality of the Wize Sniffer device with all the gas tests;
- data analysis.

Several tests have been conducted to evaluate Wize Sniffer 1.0. performances and make it ready to be integrated in the "Wize Mirror", according to a mesauring protocol, on a population of 20 subjects, of which 9 female and 11 male, containing individuals of different age (6 in RANGE1 [20-29 years]; 7 in RANGE2 [30-39 years];5 in RANGE 3 [40-49 years]; 2 in RANGE 4 [50-60 years]), habits, body type (underweight, normal, overweight).

Wize Sniffer 1.0. was able to return, on a quality level, reliable outputs in response to the several test conditions.

That is, the device has been able to detect exhaled ethanol and discriminate among different alcoholic grades, giving outputs in line with them: the voltage output rised in line with the increase in alcoholic grade. Similarly, the device output has been able to follow the trend in time of alcohol adsorption, i.e. disposal: the voltage output rised in line with the increase, in amount, of the same alcoholic drink, and decreased in time, when alcohol consumption stopped, following the alcohol disposal.

Reliable outputs were registered also during the test regarding smoking habit: the device was able to distinguish, as often as not, between smoker subjects and non-smoker subjects, as well as there was a rise in carbon monoxide and ammonia concentration values after smoking.

Interesting results were collected also during the test regarding carbohydrates metabolism: still on a quality level, hydrogen concentration values were in line with bibliography, showing in case of subjects suffering overweight problems or after smoking, and a decrease in case of exercise or after drinking alcohol.

Concerning numerical results, that is, exhaled substances concentration numerical values, some considerations have to be made. The observed concentration values corresponding to exhaled ethanol were generraly in line with the ones output from alcohol tester, even though we can not conclude that there was a complete overlapping; on the contrast, no relevant overlapping was observed when comparing with the results output from gas chromatography.

Neverthless, this is understandable, considering the different resolutions of the several instrumentation. Gas cromatography-mass spectrometry is the gold-standard for gas analysis, having very high resolution and sensitivity.

Gas chromatography is able to discriminate among several substances, one by one, with high precision and high resolution. On the other hand, our device is affected by sensors' cross-sensitivity: the presence of two multi-sensing sensor is an aspect that has not to be ovelooked. If having multi-sensing sensors may be advantageous on one hand, because it allows to detect a wider set of analytes, on the other hand it affects the selectivity of the sensors themselves.

Furthermore, a strong hypothesys was made in the model consisting of five non-linear equations system to calculate exhaled molecules concentrations: for two multi-sensing sensors, we made the hypothesis that the several contri-

butions from the several substances add together linearly. This is an aspect to be improved in the next future.
The comparison between Wize Sniffer results and the ones output from piCO+ Smokerlyzer has been rather satisfying. There was a relevant overlapping, due to the specific transduction function of both the system.
Anyway, if the lower sensitivity is the major limit of our device, nevertheless it presents numerous advantages and benefits. The table 2.11 summarizes the comparison between the Wize Sniffer and classic techniques for breath analysis such as gas chromatography.

Table 2.11: **Wize Sniffer VS Gas chromatography**- advantages and limits.

WIZE SNIFFER	GAS CHROMATOGRAPHY
Limited resolution (tied to the sensors' one)	high resolution and accuracy
Real time analysis	Time consuming (about one hour for one sample)
Portable	Not portable
Low-cost	Expensive
Its modular platform allows to change the sensors easily, according to the analytes to detect	Changing the set of detected molecules means changing the instrumentation, with high costs
Its results are easy to understand	Its results require expert interpretation

It can be recognized that this first prototype of Wize Sniffer had good performances and gave good results on a quality level, the future developments of Wize Sniffer 1.0. will aim to improve its performances, both in terms of hardware and in terms of software:

- First of all, as discussed before, the non-linear equations model used to calculate exhaled substances concentration will be reviewed in depth.

- An accurate calibration of the Figaro gas sensors might be useful for this purpose. Furthermore, making more trials, we will able to evaluate error percentages, extrapolating the average values from our results, and calculating the standard deviation.

- We'll try to evaluate also the Respiratory Exchange Ratio (RER), that is the dimensionless ratio between exhaled carbon dioxide and inhaled oxygen. Lower values of RER (about 0.7) represent the ones expected for fat oxidation, while higher values of RER (about 1) represent the ones expected for carbohydrates oxidation, allowing the amounts of fat/carbohydrates employed for energy expenditure.
  The Bod-Pod, manufactured by COSMED s.r.l. (http://www.cosmed.it/en/products/body-composition/adult-children-bod-pod-ex) will allow us to calcuate TGV, too, that is the toracic gas volume.

- Moreover, thanks to Wize Sniffer **modular platform wich allows the sensors to be inter-changebles**, we'll try to use other types of gas sensors with different working principle, then, trying to realize an hybrid platform and also increasing the number of detected molecules. To this end, our efforts will be especially focused on the detection of nitric oxide and nicotine.
  Exhaled nitric oxide is a marker of inflammatory asthma phenomena. Nowadays, gas sensors for human exaled nitric oxide are stand-alone, are very expensive, and not able to be integrated in a sensors platform like ours.
  Reliable commercially gas sensors for human exhaled nicotine are not available.
  For these purposes, our interest will be focused on polyaniline - based

(conductive polymer) electrospun nanofibres ([2]). An important aspect of nanofibers is the increasing of surface/volume ratio, which may increase sensors' sensitivity, strongly affected by the specific surface of sensing material. Polyaniline, showing high chemical sensitivity and huge porosity can be considered excellent materials for the immobilization of gas molecules.

An actual research topic is the employment of polyaniline films as sensing element for exhaled nicotine gas sensors ([**?**]).

To realize specific gas sensors for human exaled nitric oxide and human exaled nicotine using polyaniline nanofibers as sensors' sensing element, will be our future great challenge.

Appendix A - Focus on Wize Sniffer 1.0. hardware and software

Figaro gas sensors general structure

Their general structure is shown in Figure 2.18.Using thick film techniques, the sensor material is printed on electrodes (noble metal) which have been printed onto an alumina substrate. The main sensing material of the sensor element is metal oxide semiconductor (SnO_2).

One electrode is connected to pin 2 and the other is connected to pin 3 (see for example the measuring circuit shown in Figure 2.19). A metal oxide heater printed onto the reverse side of the substrate and connected to pins 1 and 4 heats the sensing material, in order to maintain the sensor at a fixed temperature.

Reading data from TGS 4161 by means of the module AM-4-4161

TGS4161 is a solid electrolyte sensor, which requires a special microprocessor provided for it to obtain an accurate measurement of CO_2 concentration. For this purpose, Figaro Engineering makes available the module

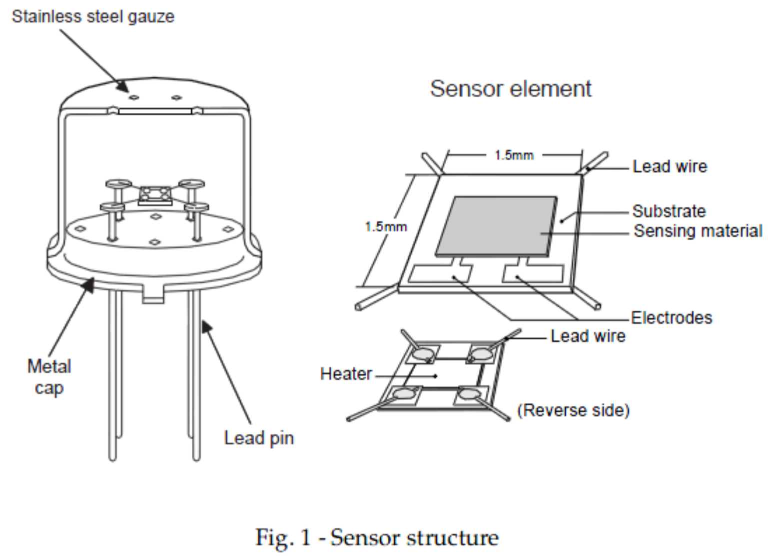

Figure 2.18: **Sensor's general structure**

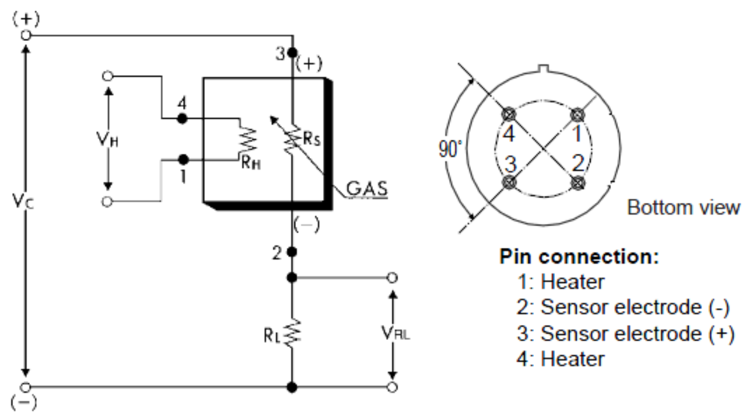

Figure 2.19: **Basic measuring circuit for TGS2620 and TGS2602**

Figure 2.20: **AM-4-4161 module for carbon dioxide sensor TGS 4161. The sensor is placed on this special microprocessor, which allows an accurate reading of carbon dioxide concentrations.**

AM-4-4161(shown in Figure 2.20).

TGS 2442 and TGS2444 particular measuring circuit

These two sensors require a basic measuring circuit like the one shown in Figure 2.21.Sensor's resistance is calculated as for TGS2602 and TGS2620, it means, by a voltage divider.

Application of a Vc pulse condition is required to prevent possible migration of heater materials into the sensing element material. Under extreme conditions of high humidity and temperature, a constant Vc condition could result in such migration and cause long term drift of Rs to higher values.
A Vc pulse results in significantly less driving force for migration than a constant Vc condition, rendering the possibility of migration negligibly small.
For TGS2442 the heating/Vc cycle is the one shown in Figure 2.22, while the one for TGS2444 is shown in Figure 2.23. How the pulsed Vc has been applicated will be explained in the following sections.

Temperature and humidity sensor Sensirion SHT11

The Sensirion SHT11(shown in Figure 2.24) has been placed in store cham-

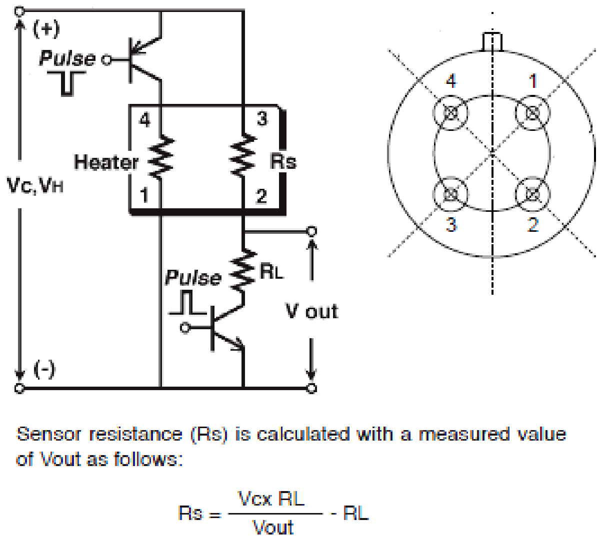

Figure 2.21: **Basic measuring circuit for TGS2442 and TGS2444.**

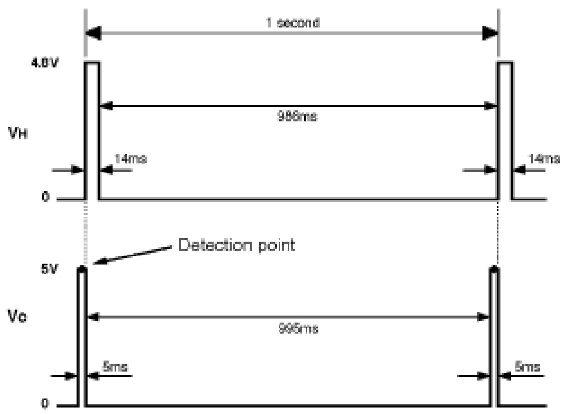

Figure 2.22: **TGS2442 heating/Vc cycle. For achieving optimal sensing characteristics, the sensor's signal should be measured after the midpoint of the 5ms Vc pulse of 5.0V**

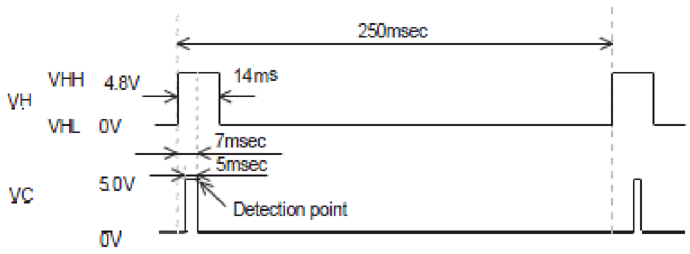

Figure 2.23: **TGS2444 heating/Vc cycle. For achieving optimal sensing characteristics, the sensor's signal should be measured after the midpoint of the 5ms Vc pulse of 5.0V**

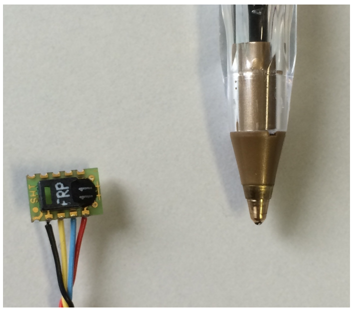

Figure 2.24: **Sensirion SHT11.The figure above shows the very small dimension of this sensor.**

ber, as shown in Figure 2.25.

Sensirion SHT11 has a humidity accuracy of about +/-3.5% and a temperature accuracy of about +/-0,5 °C @ 25 °C. Its scheme is shown in Figure 2.26; note that it has a digital output, in contrast to the other sensors (the gas ones) which have an analogue output.

Multiplexer CD4051B, Texas Instrument

A scheme of CD4051B is shown in Figure 2.27.

Buffers LM124-N, Texas Instrument

A scheme of LM124-N is shown in Figure 2.28.

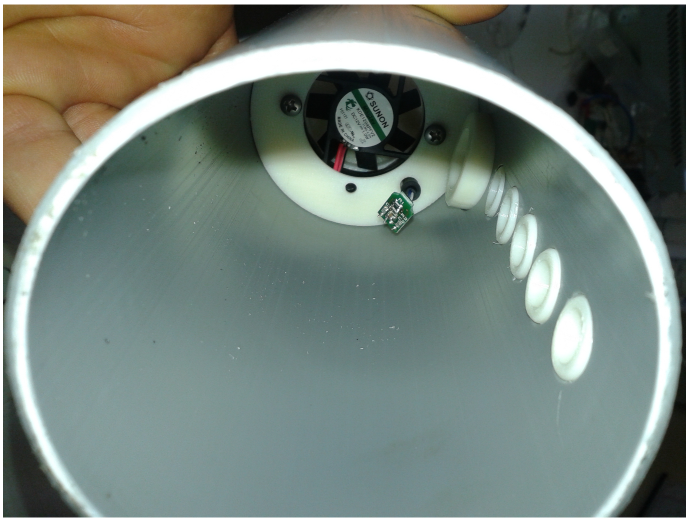

Figure 2.25: **Sensirion SHt11 has been placed in the immediately chamber's entrance**

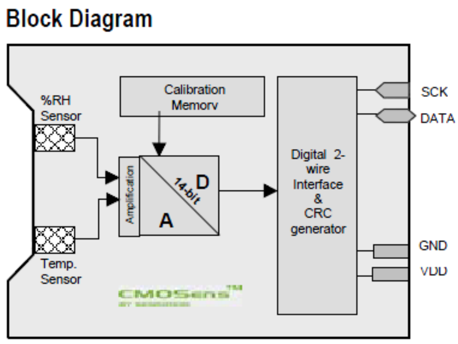

Figure 2.26: **Sensirion SHT11's scheme**

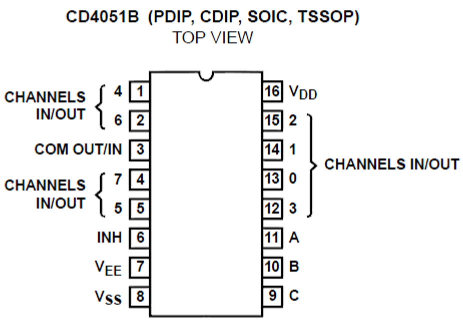

Figure 2.27: **Multiplexer CD4041B manufactured by Texas Instrument**

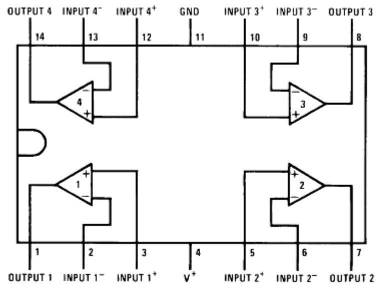

Figure 2.28: **Buffer LM124-N manufactured by Texas Instrument**

Reading the values from the sensors- the code implemented on Arduino Ethernet board

The loop code on Arduino Ethernet board necessary to read the values coming from the different sensors (Figaro gas sensors, COSMED gas sensors and Sensirion temperature and humidity sensor) is shown below as pseudocode:

Gnuplot to fit the sensors' sensitivity curves

Figro sensors' sensitivity characteristics map the ratio Rs/Ro as a function of the substance concentration (see Chapter 1). In particular, these sensitivity characteristics derive from a power low such as:

$$\frac{Rs}{Ro_{TGSxxxx}} = A_{TGSxxxx_{sub}}[sub]^{\alpha_{TGSxxxx_{sub}}} \tag{2.8}$$

The constant parameters $A_{TGSxxxx_{sub}}$ and $\alpha_{TGSxxxx_{sub}}$ are different for each sensor. They has been calculated fitting the bilogarithimc curves representing the sensitivity characteristics. For this purpose, the software "Gnuplot" has been used (see Figure 2.30 and Figure 2.31).

- ✓ Out of the loop, 10 variables “float” are declared
  - ❖ *float “out(i)”;*
- ✓ Setting the multiplexer control inputs (Arduino’s pin7, pin2, pin4) according to the input to read es:

```
❖ digitalWrite(7, LOW); //setting mux control input (0,0,0) to read mux input0
  digitalWrite(2, LOW);
  digitalWrite(4, LOW);
```

- ✓ The gas sensors’ output is sent in input to Arduino analogue pins; so, to read them:

```
❖ out(i) = analogRead(i)*0.0049;
```

- ✓ For the TGS2442 it was necessary to set the digital pins to heat and sense the sensor itself by a pulse heating/powering cycle (as described in the section referring to the hardware-gas sensors). For the TGS2444, on the contrary, this was not necessary, so, it was powered with a continuous power source, as for the others Figaro sensors.

```
❖ digitalWrite(3, LOW);
   analogWrite(9, 245);
   delay(14);
   analogWrite(9, 0);
   delay(981);
   digitalWrite(3, HIGH);
   delay(3);
   out0 = analogRead(0)*0.0049; //0.0049 is the bits' resolution in Volt
  digitalWrite(3, HIGH);
  digitalWrite(3, LOW);
```

- ✓ For the Sensirion SHT11 it was necessary to load its libraries to read it:

```
❖ #include <SHT1x.h> //for SHT11 (humidity and temperature sensor)
  #define dataPin 6
  #define clockPin 5
  SHT1x sht1x(dataPin, clockPin);
  temp_c = sht1x.readTemperatureC();
  humidity = sht1x.readHumidity();
```

Figure 2.29: **The pseudocode of the program implemented on Arduino Ethernet board to read data from the sensors.**

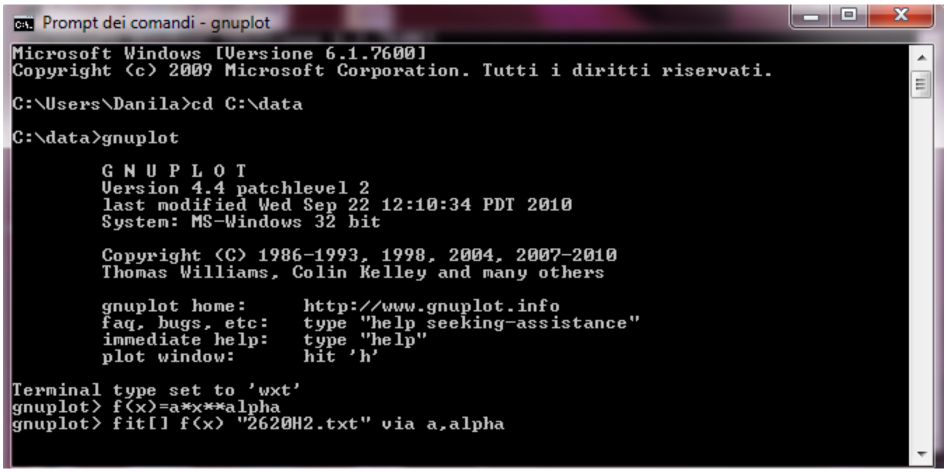

Figure 2.30: **The power low function $f(x) = A(x)^{\alpha}$ was inserted in the command lines, just as the file containing the coordinates of the sensitivity curve's points.**

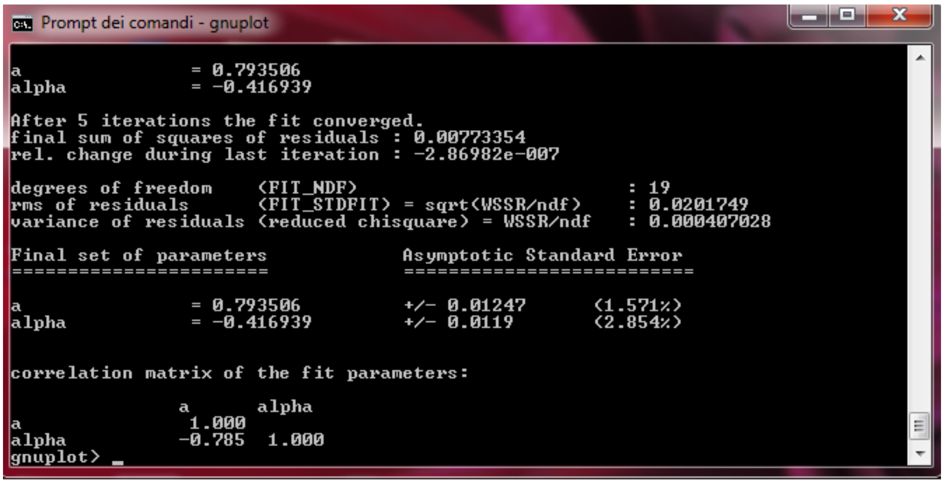

Figure 2.31: **The constant parameters $A_{TGS2602_{Eth}}$, $\alpha_{TGS2602_{Eth}}$ resulted from curve fitting. Note the very low asymptotic standard error.**

Bibliography

[1] Dongmin Guo, David Zhang, Naimin Li, Lei Zhang, Jianhua Yang, 2010 *A Novel Breath Analysis System Based on Electronic Olfaction*, IEEE Transactions on Biomedical Engineering

[2] Mario D'Acunto, Antonio Benassi, Federica Chiellini, Danila Germanese, Randa Ishak, Massimo Magrini, Emanuele Pagliei, paolo Paradisi, Marco Righi, Ovidio Salvetti, 2013 *Wize Sniffer: a new portable device designed for selective olfaction*, Proceedings of SUPERHEAL2014 conference, Angers, France

[3] D.G. Thompson, P. Binfield, A. De Belder, J. O'Brien, S. Warren, 1985 *Extra intestinal influences on exhaledbreath hydrogen measurements during the investigation of gastrointestinal disease*, Gut