UNIVERSITAT DE BARCELONA

Final Degree Project
Biomedical Engineering Degree

Development of a Breath Sampler and proof of concept

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Abstract

In the last few decades, there has been a pervading interest in non invasive technologies for diagnosis, monitoring as well as for treatment in the medical field. As part of this trend, breath analysis has emerged, and although very promising, there is a main issue this field's development faces, the lack of reproducibility and reliability of protocols since there's no standardization in the sampling process.

This project aims to develop a Breath Sampler focused on achieving a protocolized sampling methodology that allows the collection of the fraction of exhaled air that has been in contact with the alveoli and therefore is rich in metabolites. To do so, different improvements of hardware and software are implemented on a first Breath Sampler prototype and a proof of concept is carried out to verify that itoperates as intended.

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1. Introduction

1.1. Motivation

This project was chosen due to a personal interest in carrying out a multidisciplinary project that allowed me to learn from different fields and join them together towards a common end and develop a medical device prototype.

Knowing that it would give me an overview of the development process of a non-invasive diagnostic and monitoring tool that could be applied in medical facilities made this project specially interesting, also having in mind the fascinating field that breath analysis is and the future potential it has.

For all this, the development of the breath sampler was the perfect opportunity to expand my knowledge and do hands-on work in a project that was really appealing.

1.2. Objectives

Breath analysis seems to be a promising diagnostic tool for the screening of patients affected by a wide range of diseases, as well as for the monitoring of physiological processes in a low invasive way, allowing to monitor in real time the concentrations of breath markers. Notwithstanding, it is noteworthy the lack of standardized procedures for breath tests.

Being framed in this context, the main objective of this project is to provide a tool to protocolize the obtention of exhaled breath samples to diagnose patients in a non-invasive way by developing and improving an electronic device to obtain samples of exhaled air from patients.

A breath sampling device is therefore proposed in order to obtain probes of exhaled air, particularly from the deepest part of the lungs, from which the identification and quantification of potential disease biomarkers can be performed.

For this purpose, the following objectives will need to be achieved:

- Review of current literature on breath analysis and breath sampler devices
- Development of a hardware system and design of a compact case structure to put together a cost competitive breath sampler device
- Implementation of software to be used through an user friendly interface in order to achieve the desired functionalities for the hardware
- Development of a complementary device to obtain environment air samples to be used as a reference for breath sampler's samples analysis
- Performing a proof of concept of the breath sampler

1.3. Methodology and report structure

First of all, it must be mentioned that previous work of a first breath sampler prototype already existed. Therefore, to acquire the necessary knowledge for understanding and planning the project, all documentation with this regard was read and a bibliographic research was carried out.

Also, as part of the familiarization process with the project, the task of developing a alcoholmeter with an Arduino Uno microcontroller board and an etanol MQ3 sensor was conducted.

After that, it was planned to take up the breath sampler prototype, perform a detailed analysis of its components and implement any necessary updates and changes to make it functional.

The first task to arise came from the necessity to have all components connected between themselves and with the governing microcontroller in a sturdy and compact way at the same time that it would allow the exchange of components in case of malfunction or brokenness. For this purpose, after learning the required bases for electrical circuit design, an Arduino shield PCB (Printed Circuit Board) was designed and assembled.

After that, a more mechanical phase started with the objective of assembling all the hardware components and casing the prototype. To do so, and create a tailored case as well as a tubing part that was required, self-directed learning on CAD (Computer Aided Design) and 3D printing was carried out.

At this point, the first test of functioning was attempted but failed. Several tests concluded in the inference of an insurmountable incapacity to achieve vacuum as needed for the correct functioning of the device as it will be explained later in detail. This implied a need of a new hardware design for the prototype, as well as software, PCB and 3D printed case updates as a result of this. Also, new implementations onto the software were done to improve the original functionality planned for the breath sampler.

Simultaneously, an environment sampler device was designed and developed in order to acquire ambient samples of the air so in the moment of analyzing the components present in the samples from the patient, a reference of the environment's composition is available for comparison.

Finally, a series of trials were performed in the search of evidence to demonstrate the feasibility of the device performing a proof of concept.

All the project is performed in the Electronics Department at the Physics Faculty from Universitat de Barcelona (UB).

1.4. Scope and Span

As a Final Degree Project, limitations related with time and cost must be considered in the present document, as well as the fact that the breath sampler is designed to be implemented into the clinical field for the diagnosis and monitoring of different diseases, and therefore some regulations and considerations must be present along its construction.

At the beginning of the project, no cost limitation was put into consideration as the components of the first prototype were available for use. Nevertheless, after consideration of the hardware redesign of the device, new components had to be acquired, and it was kept in mind that this limitation could affect the hardware of the device, being unable to design the best version. Being the main goal to keep the breath sampler a cost competitive and portable device, this constraint was already planned. This was also a consideration when

deciding to opt for 3D printing for the manufacturing of the case, as well as the before mentioned portability factor, playing a fundamental role in the selection process of the new components and its assembly.

Regarding to the time limitation, the project was planned to last a slightly over a semester. The knowledge on CAD modeling, PCB design and assemblying as well as in Labview, the software in use for the programming of the project, was limited, so it was expected to obtain a functional prototype that could nevertheless be further improved in the future. Also, a proof of concept with a group of real patients was kept in mind as a means to test the actual use of the device, as well as a more in depth analysis of the samples taken with specialized equipment, but due to the COVID-19 situation along with the time limitations, and the available resources in the Electronics Department, it has been kept out of scope.

Another limiting factor typically to be taken into account while developing a medical device, is the dimension and weight, as some of the medical apparatus that are commonly found in a hospital, such as a magnetic resonance machine, take up a lot of space and are heavy, but in this case, the device is designed to be small and portable and so are all the components, so no other limitation is added. There was all the necessary space to work in the Laboratory of the Department, and the tools required and supplies were found here as well as in the workshop of the department.

The scope of the project is to create a breath sampling device that collects samples of the exhalation of a patient at its ending. The reason for this is that metabolites released as a result of normal activity or pathological disorders, are eventually either metabolized or excreted, and one of the vias for this is exhalation, comprising an opportunity for non invasive diagnosis or monitoring through the analysis of this metabolites.

The expected end result is a functional prototype of a breath sampler, packed up in a tailored 3D printed case along with a complementary environment sampling device that is proved to allow the collection of exhaled air that could be further improved and applied in different medical fields.

2. Background

2.1. State of the situation

This project emerges from the need of standardization of breath sampling collection with the purpose of acquiring information on the clinical state of an individual by detecting metabolic compounds present in exhaled breath.

Previously in the Electronics Department, over the academic year 2019-2020, Mrs Maria Bel Bordoy Pont worked on the development of the breath sampler prototype as her final degree project, being based on previous work by Mrs. Umi Mir Kato. In this period of time and under the supervision of Dr. Antonio Pardo, she continued the project implementing improvements on both hardware and software, but due to the pandemic situation, it could not be tested.

It is also worth stressing that the Electronics Department has grown a trajectory over time in the processing and analysis of breath samples as well as intestinal gas aside from this current breath sampling device, alongside with the study of putative biomarkers for chronic obstructive pulmonary disease (COPD), which is fundamental for a reliable final application of the breath sampler.[1, 2]

It is consecutively presented a summary of the state to understate the starting point of this project, focusing on the first prototype developed.

This first prototype is based on the system designed in Salvo et al.(2015) [3] which consists of two parts, an analysis unit and a sampling unit as seen in the figure right below.

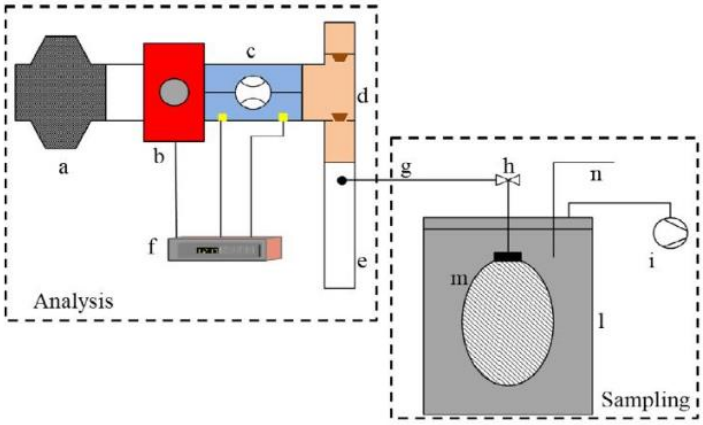

Figure 1. Schematic diagram of the reference breath sampler. (a) mouthpiece, (b)CO2 sensor, (c) flow meter, (d) valve, (e) collection chamber, (f) Mercury module, (g) connection tube, (h) solenoid valve, (i) vacuum pump, (l) air container, (m) Nalophan bag, (n).

The hardware resembles the original assembly of the reference breath sampler, although some changes are applied. On the other hand, the analysis part raises some changes when performing the comparison.

The mercury mode and CO_2 sensor that can be found in the reference system's analysis unit are not implemented due to the limitations they are connected to, being these a non suitable prolonged time response of the sensor, and an elevated price point.

Conversely, the sampling section owns a methacrylate vacuum chamber that hosts a Tedlar bag used as recipient to store the samples. It can be also noted that a second solenoid valve is added onto the design of this unit.

All electrical components are connected using a PCB and a lab bench power supply or a USB type B 2.0 tether is used to give the power needed to run all electronics and equipment. Moreover, an Arduino Uno microcontroller board is programmed to interact with the electrical circuit.

To control all the components, a Labview program with a user interface is designed. Arduino interacts and controls them thanks to an interface with common embedded platforms that is available to deploy LabView code to run on Arduino. This interface is known as LINX and it is an open source project by Digilent. Also, the data generated by the peripheral sensors to Arduino, is read, plotted and can be saved in a file.

2.2. State of the art

In order to get a better understanding of the operability of the device, it is a fundamental requirement to understand the context and frame of its future application in the clinical field. Therefore, an overview of the respiratory system will be displayed hereafter.

In addition, the state of the art of breath sampling devices is reviewed and different metabolites and volatile organic compounds (VOCs) that can be detected in breath are looked into, to finally discuss the different analytical methods that could be used to identify these compounds into breath samples collected by the before mentioned devices.

2.2.1. General view of the respiratory system

The respiratory system consists in an intricate network of specific organs and structures that allow gas exchange in the human body, with the primary purpose of equalizing the partial pressures of the respiratory gases in the alveolar air with those in the pulmonary capillary blood. [4]

Structure wise, the system can be divided into two different parts denominated upper and lower airways respectively. Th supper part includes the nose, with nasal cavity, frontal sinuses, maxillary sinus, larynx, and trachea; and the lower tract includes the lungs, bronchi and the alveoli.

The respiratory process is possible thanks to three functions: ventilation, diffusion and perfusion.

The ventilation consists of:

1. Inspiration, which is the expansion of the chest with a negative intrapulmonary pressure when air flows into the thorax.

2. Expiration, when the intrapulmonary pressure is higher than the atmosphere, air will flow out of the lungs.

Then, diffusion is the process whereby gases move from an area of high pressure to low pressure. This includes the internal respiration, understood as the movement in the internal tissues between cells and capillaries, and the external respiration, when gas is exchanged between the alveoli and lung capillaries.

Perfusion refers to the passage of blood flow through the circulatory system to an organ or a tissue. Alveoli are perfused by capillaries so the diffusion of oxygen and carbon dioxide can take place and the required equalization of partial pressures of the respiratory gases before mentioned is achieved.

The alveolar pressure is a fundamental factor in the respiratory process, meaning the pressure inside the lung alveoli, which in general terms equals the atmospheric pressure when the glottis is open, and no air is flowing into or out of the lungs. [5]

During the ventilation function of the respiratory system in order to achieve an inward flow through the airways into the alveoli, pressure must fall slightly below atmospheric pressure values. This slight negative pressure is enough to make possible the pull of 0.5 litres of air into the lungs in the 2 seconds generally required for normal quiet inspiration. During expiration, the opposite occurs.

The rate at which new air reaches these areas as part of the ventilation process is called alveolar ventilation, but not all inspired air reaches them, and a fraction of it just remains replenishing the airways. These sections where no gas exchange occurs are the so called dead space, not being useful for gas exchange, and it is this air that is expired in the first place in the expiration phase of the ventilation. [6][7]

As it can be extracted from this, alveolar ventilation is one of the major factors determining the concentration of the different compounds that can be found in the alveoli, such as oxygen, carbon dioxide, other metabolites and volatile organic compounds, defined by the European Environment Agency (EEA) as “Organic chemical compounds that under normal conditions are gaseous or can vaporise and enter the atmosphere.”[8]

This air found in the alveoli, differs in composition with regards to atmospheric air, due to the following facts:

- As explained before, alveolar air is just partially replaced by atmospheric air with each breath.

- Oxygen is constantly being absorbed into the pulmonary blood from the alveolar air while carbon dioxide is constantly diffusing from the pulmonary blood into the alveoli.

- Along its wat through the airways in the inhalation phase of the respiration, atmospheric air is humidified.

Additionally, a comparison among athmospheric air, inhaled and exhaled air can be performed. While inhaled air is similar to the atmospheric air, exhaled air contains different compounds that are captured at the tissue-air interface along the respiratory tract as well as carbon dioxide from cellular activity, and lacks oxygen in comparison to the inhaled air due this same reason and thanks to the diffusion phenomena. Most of these compounds are metabolic products, so the exhaled breath contains cellular activity footprints.

	Atmospheric Air* (mm Hg)		Humidified Air (mm Hg)		Alveolar Air (mm Hg)		Expired Air (mm Hg)	
N_2	597.0	(78.62%)	563.4	(74.09%)	569.0	(74.9%)	566.0	(74.5%)
O_2	159.0	(20.84%)	149.3	(19.67%)	104.0	(13.6%)	120.0	(15.7%)
CO_2	0.3	(0.04%)	0.3	(0.04%)	40.0	(5.3%)	27.0	(3.6%)
H_2O	3.7	(0.50%)	47.0	(6.20%)	47.0	(6.2%)	47.0	(6.2%)
TOTAL	760.0	(100.0%)	760.0	(100.0%)	760.0	(100.0%)	760.0	(100.0%)

*On an average cool, clear day.

Figure 2. Partial pressures of respiratory gases as they enter and leave the lungs at sea level. Difference in gas concentration between athmospheric and alveolar air.[4]

2.2.2. Breath sampling

The analysis of biomarkers in exhaled breath has captured increased interest in the clinical field because of their potential application in pulmonary diagnostics. Composition wise, exhaled breath consists of two components. The first 150 mL is dead space air and the remaining 350 mL, known as alveolar breath, comes from the lungs, where gaseous exchange between blood and air occurs, being primarily a mixture of nitrogen,oxygen, carbon dioxide, water, and inert gases and the remaining fraction more than one hundred volatile organic compounds (VOCs) in the parts-per-million (ppm) to parts-per-trillion (ppt) concentration range.

The compounds found in breath vary qualitatively and quantitatively between individuals especially for VOC, and only a few are common including isoprene, acetone, ethane, and methanol. Inorganic gases are also endogenously produced, for example, nitric oxide (NO), carbon monoxide (CO) and carbon dioxide (CO2) are commonly found in breath.[9]

Keeping into account that these molecules represent products of core metabolism, their measurement can potentially provide important diagnostic information, shedding light on the physiologic significance of this substances in breath is essential for their analysis and identification as breath biomarkers. [10][11]

The performance of breath tests with medical diagnostic and monitoring objectives constitutes a noninvasive, easily repeatable test, that is notassocia ted with any discomfort nor patient counter-indication. Moreover, it is also an in-expensive way to rapidly screen for certain diseases. Also, exhaled breath content carries various biomarkers of respiratory function that are not present in other metabolization or excretion via for metabolites such as in serum or urine. [12]

But in order to turn breath analysis into a new gold standard for the diagnosis and monitoring of several diseases, there are a few hurdles. First of all, a more in-depth understanding of the relationship between diseases and breath biomarkers is required. Secondly, a normalized set of procedures for sampling, and analysis is lacking. And finally, standardization of exhaled breath content data remains a difficult but necessary challenge to overcome before breath markers can be used in the clinical diagnostics.

Even though a few obstacles remain in the way, the use of breath tests for diagnosis of pulmonary diseases shows great promise as new developments and improvements arise.

2.2.3. Breath metabolites

VOCs, being a wide range of carbon-based chemicals emitted by the human body often can reflect the metabolic condition of a subject, and due to this, their analysis is a non-invasive tool to assess information about health status.

As explained before, VOCs are found in exhaled breath, but they can also come from the external environment and be inhalated by the subjects. For this reason, the study and characterization of VOCs is fundamental in the field of breath analysis.

From this necessity, arises breathomics, a special branch of metabolomics that quantifies volatile organic compounds from collected exhaled breath samples. It aims to understand how breath molecules relate to diseases, mechanisms and bilological pathways.

It is of special interest the Human Breathomics Database (HBDB) [13], created to provide organized breath VOCs related references and biomedical information.

So far, the database contains a total of 913 VOCs in relation to human exhaled breath research reported in 2766 publications, being the most comprehensive HBDB of VOCs in human exhaled breath to date. It is a useful and organized resource for researchers and clinicians to identify and further investigate potential biomarkers from the breath of patients.

Compound	Related diseases	CAS number
Urea	Asthma, Cystic Fibrosis	57-13-6
8-isoprostane	Asthma, COPD, Heart failure	155976-51-5
Toluene	Lung cancer, smoking related	108-88-3
Glutathione	Asthma	70-18-8
Hydrogen peroxide	Asthma, Bronchiectasis, COPD, Bronchiectasis	7722-84-1
Hexanal, 2,2-dimethyl-	Lung cancer, breast cancer	996-12-3
Leukotriene B4	Carcinoma, Lung Neoplasms, Pneumoconiosis, Sleep Apnea Syndromes, Asthma, Cystic Fibrosis COPD, Asthma	71160-24-2
Nitric oxide	Asthma, Asbestosis , Cystic Fibrosis, Obstructive Sleep Apnea, Obesity	10102-43-9
2-Butanone	Lung cancer	78-93-3

Acetone	Cystic Fibrosis	67-64-1
adenosine 5'-monophosphate	Asthma, Cystic Fibrosis	149022-20-8
Cyclohexane	COPD, AECOPD	110-82-7
2-metylhexane	COPD, AECOPD	591-76-4
2,4-dimethylheptane	COPD, AECOPD	2213-23-**2**
2,6-dimethyloctane	COPD, AECOPD	2051-30-1
Ammonia	Chronic Kidney Failure	7664-41-7

Table 1. List of VOCs found in breath. [14][15][16][11][17][18][19][20] [21][22][23][24]

2.2.4. Breath analysis methods

The detection of chemicals in human breath pipeline has traditionally required three steps: isolation or pre-concentration of the biomarker chemicals, separation of the biomarker chemicals from the complex background of all breath metabolites, and detection or identification of the biomarkers that are deemed important.

- Gas Chromatography - Mass Spectrometry: GC/MS is nowadays the most widely used technology for VOC analysis in exhaled breath, which is a highly sensitive and reliable technique. This technique is used mainly in offline breath analysis, as it combines the resolving power of a chromatograph with the specificity of a mass spectrometer.

  In GC/MS, the compounds present in exhaled air are fragmented into ions with different mass to charge ratios (m/z) that are detected. Unknown compounds can be identified by matching full mass spectrum of unknown peaks with a mass spectra library or database. The GC-MS technique is not only very reproducible, but it has high sensitivity and robustness for individual VOCs.[11][20]

- Electronic nose: eNoses are an assembly of nonspecific sensors which respond to different odors by producing a complex signal. They have as advantage the fact that they are cost-competitive, easy to use, provide more rapid results, low operating costs, and great portability along with flexibility in the sensor array, which can be adjusted for specialized applications. However, eNoses can not identify individual compounds in complex mixtures. [25][26][27]

- Direct mass spectrometry with different ionization processes. Currently, online real-time breath analysis is emerging through technologies based on mass spectrometry, such as PTR-MS (proton transfer reaction mass spectrometry) and SIFT-MS (selective ion flow tube mass spectrometry).

  Both instruments are based on the same three components: an ion generation zone, a reaction zone and a detection zone. Reagent ions H3O+ (both instruments), NO+ and O2+ (SIFT-MS only, with positive ion source) are generated and injected into the reaction zone, where they chemically ionize the analytes to form product ions. All ions are then analysed by a mass spectrometer (MS), usually a quadrupole-MS, separating the ions by their m/z ratio and then counting the number of ions hitting the multiplier. There are two main

differences between the two instruments: First, they differ in the way the reagent ions are generated and second, whether the ions are reacting with the analyte in a drift-tube vs. in a flow-tube. [28]

- Nanomaterial-Based VOC and Gas Sensors: Combining nanomaterials with organic recognition elements result in transducers that can be used as sensors with specific receptors that have high sensitivity and selectivity or as semiselective sensors that are less sensitive and less selective but are more suitable for characterizing complex and unknown samples. They therefore constitute a promising candidate for monitoring human breath in a noninvasive, real-time way.[29]

- Laser spectroscopy can provide real-time detection of a single or several volatile compounds, eliminating the need for collection and storage. Furthermore, this allows breath-cycle resolved sampling, resolving the different respiratory phases and continuous measurements of the VOCs present in these different phases, and the possibility to couple and validate physiological and gas exchange models. The high precision, absolute accuracy, and breath-cycle resolved sampling allow much more data to be collected and detailed biomarker studies to be performed.[30][31]

3. Market analysis

Although breath analysis has been in the spotlight as an appealing diagnosis method for a time now, being breath the one biological fluid that can be obtained noninvasively, issues related to separation and identification of discrete breath components had been slowing down the development of br[31]eath samplers until these last decades, when analytical methods have progressed enormously.

The first patents referring to an “Apparatus for analyzing the alveolar air from the lungs” date back to 1928 and they were modeled with fluid mechanics as their base, as no electric components nor even software existed at the time. Hardware and software experienced great advantages, and in 1971, Linus Pauling used gas chromatography (GC) to successfully identify hundreds of breath VOC (ranging in size from C2 to greater than C20) present at picomolar concentration.

Nowadays, non-invasive diagnostic technologies are on the rise, and not only breath samplers such as the ReCIVA Breath Sampler from Owlstone Medical, which collects volatile organic compound and respiratory droplet samples from exhaled breath to diagnose of cancer, inflammatory and infectious diseases are being developed. [32]

Also, the great potential of the principles in which this diagnose is based have drawn attention as a testing methodology to be developed in order to achieve a fast screening tool to fight against the expansion of the COVID-19 pandemic that we are living in.

A breath test developed by NUS spin-off startup Breathonix designed to detect Covid-19 and give accurate results within one minute has been developed, and has already received provisional authorisation from Singapore's Health Sciences Authority (HSA).[33]

This new Covid-19 Breath Test System is performed by an individual who simply exhales into a disposable one-way-valved mouthpiece that is connected to a breath sampler, where an integrated mass spectrometer analyses the VOCs in the exhaled breath, constituting a “signature”, different from someone who is ill.

The total time from breath sampling to results takes less than a minute, and so far, it has underwent clinical trials at three locations from last June to April, achieving a promising sensitivity of 93% and specificity of 95%.[34][35]

It can be mentioned that if differs from the breath sampler in the fact that collects samples for its analysis in-place instead of off-line and not discriminating any particular VOC or selection of them of interest as it would be expected to do with the samples of the Breath Sampler prototype.Still, this constitutes a very promising advancement in the breath analysis field, that shows that there is plenty of room for research and improvement in this field with great potential for the near future.

4. Regulatory and legal issues

The fruit of the present project 's labour will be considered a prototype, so no medical device regulations apply to it yet. However, for its future development, some legal aspects must be taken into account.

Firstly, being produced in Spain, it must follow the Spanish Medical Device Regulations and Classification. The organization responsible of this regulation is the Spanish Agency of Medicines and Health Products (AEMPS) applying the Directive 93/42/EEC. [36]

In Spain, all medical products are classified depending on the specified criteria found in the annex IX of the Real Decreto 1591/2009, according to the EU risk-based classification system. Each medical device is included in one of these types of agreements according to the degree of danger or risk in their use, so type I are considered low risk devices, those of IIa class of low medium risk, those of the class IIb of medium high risk and those of class III of high risk. The device of this project would be classified as a class IIa, as this device is introduced in a body orifice without remaining in it.

If the product were to be commercialized in Europe, it would have to undergo a conformity assessment to demonstrate that it meets legal requirements to ensure its safety and performance as intended. This conformity assessment usually involves an audit of the manufacturer's quality system. After the medical device has gone through the conformity assessment, manufacturers can place a CE (Conformité Européene) mark.

Finally, ISO and IEC standards must be also fulfilled. The ones to be applied io the breath sampler would be ISO 13485, that defines safety and quality in the medical industry, and IEC 60601, including a series of technical standards for the safety and essential performance of medical electrical equipment.

5. Concept engineering stage

As mentioned before, the starting point of the project is an existing first prototype of a breath sampler, and due to this, the concept engineering is strongly related to it.

Hereunder the overall project definition, project planning, development processes and possible alternatives are studied, concluding into a final proposed solution.

5.1. Hardware

The hardware in the final prototype has as a base the first prototype, that at the same time is referenced to the system designed in Salvo et al.(2015). Although it was not part of the scope of the project at the beginning, a redesign was required, but most of the components of the first device prototype were used because of availability purposes as well as to keep the project inside the budget and time limitations. Some research is also done in order to provide some considerations for the development of a future final device.

5.1.1. Microcontroller

A microcontroller is an integrated circuit that contains a microprocessor, a computer processor contained on an integrated-circuit chip, along with memory and associated circuits and that is designed to perform the specific tasks of embedded systems.

The general microcontroller consists of the micro processor, the memory (RAM/ROM/EPROM), Serial ports, peripherals (timers, counters), etc. on a single chip.[37]

The first prototype of this project was implemented using an Arduino Uno, a microcontroller board based on the ATmega328P [38]. Being Arduino an open-source electronics platform based on easy-to-use hardware and software, its use presents strong advantages such as its inexpensiveness, a simple, clear programming environment, and extensible software and hardware. Nevertheless, with this mentioned open source advantage comes a limitation for a final device, as it not a very suitable option to implement when releasing a medical device into the market, which has to be regulated, and nowadays there are still many challenges for open-source hardware to comply all regulatory issues and certifications.

A second controller board was used for the ambient air accessory sampler. Due to its availability at the laboratory, an ELEGOO UNO R3, a generic model of the previously described Arduino Board was implemented.

It can be mentioned that this is an add-on for the purpose of performing a more robust sample data analysis by acquiring data from the testing environment too, but because it is not in touch with the patient, no regulatory issues would arise by its use, and the controller board used would still be suitable.

5.1.2. Sensors

The choice of the device's sensor is one of the most important factors in order to achieve a functional and reliable breath sampler. Researching the literature about this topic, two types of sensors prevail: flow and CO_2 sensors.

- **Flow sensor**

A flow sensor, also referred to as a "flow meter", is an electronic device that measures linear, nonlinear, volumetric or mass flow rate of a liquid or a gas within pipes and tubes. Its objective is to quantification bulk fluid movement. [39]

In the prototype's case, its function consists in the detection of the inhalation or exhalation phase of the subject performing the test through a flow trigger.

Flow can be measured in a variety of ways, and the type of flowmeter used depends of the field of its application.

In the medical field, for example in CPAP devices, anaesthesia equipment or other respiratory devices where gas flow in the respiratory process needs to be measured, thermal mass flowmeters can be found. They monitor variations in one or more of the thermal characteristics (temperature, thermal conductivity, and/or specific heat) of gaseous media to define the mass flow rate. [40]

To measure, they use combinations of heated elements and temperature sensors to measure the difference between static and flowing heat transfer to a fluid and infer its flow with a knowledge of the fluid's specific heat and density. The fluid temperature is also measured and compensated for. [41]

They introduce a known amount of heat into the flowing stream and measure an associated temperature change, or maintain a probe at a constant temperature and measure the energy required to do so.[42]

Model Type		Company Connection Material			Price (EUR)
SFM3000	Mass flowmeter	Sensirion	I2C bus	PPE+PS, Si,Si3N4, Gold, Epoxy, Polyurethane	123
SFM3100	Mass flowmeter	Sensirion	I2C bus	PPE+PS blend, Si, Si3N4, Gold, Epoxy, Polyamide, silicone, FKM	147,54
SFM3200	Mass flowmeter	Sensirion	I2C bus	Si, Si3N4, Gold, Epoxy, Glob Tob, PPSU, Polyurethane, stainless steel	153,96

Table 2. Flow sensor comparison.

- **CO_2 sensor**

It is found in literature that CO_2 sensors are typically used in clinical applications due to the fact that this gas, produced during the respiration, is the most easily detectable component of exhaled breath. Therefore, this gas is an adequate parameter to monitor the breathing process and exhaled air sample collection.

Generally in the medical field, the CO_2 sensor is considered mainstream if inserted in the tubing part of a device that continue the patient's airways circuit towards a device, quantifying the gas as it goes through it; or sidestream, when the sensor is found within the equipment.

Some examples of CO_2 sensors are summarized in the following table [43][44][45]

Model Company		Connection Technology Response time			Price (EUR)
Capnostat 5	Respironics	Standard – Lemo Redel 8-pin plastic	Non-dispersive infrared (NDIR) single beam optics, dual wavelength	T < 60 ms	540
MH-Z14A NDIR CO2 Module	Winsen	Serial Port (UART), PWM or Analog output (DAC)	NDIR	T90 < 120s	20,30
SCD30	Sensirion	UART and I2C interface	NDIR	T63 < 20s	52,99

Table 3. C02 sensors comparison.

5.1.3. Valves

Keeping into account that just a fraction of exhaled air is aimed to be collected, a gateway component is needed to let pass through and into the sample collection bag just the exhaled breath of interest.

For this purpose, a normally closed valve is implemented, to be opened when the triggering condition programmed is met, allowing the flow to go through and deflecting it otherwise.

Solenoid valves are the most frequently used control elements in fluidics.They are electromechanically-operated and offer fast and safe switching, high-reliability, long service life, good medium compatibility of the materials used, low control power and compact design. These characteristics make them widely used in different types of equipment in the context of the medical field.[46]

5.1.4. Pumps

The container in which the sample is stored until it is extracted to be analyzed after going through the sensor and the valve is placed inside a chamber where vacuum is generated thanks to a pump.

There are different types of pumps, such as positive displacement pumps, impulse pumps, velocity pumps, gravity pumps or steam pumps.

For this project, it is wanted that the fluid is gets trapped, and forced to move into a discharged pipe, which is the principle of functioning of the positive-displacement pumps. Inside this group,diaphragm pumps are found. [47]

They use a combination of the reciprocating action of a rubber, thermoplastic or teflon diaphragm and suitable valves on either side of the diaphragm (check valve, butterfly valves, flap valves, or any other form of shut-off valves) to pump a fluid.

When the volume of a chamber of the pump is increased (the diaphragm moving up), the pressure decreases, and fluid is drawn into the chamber. When the chamber pressure later increases from decreased volume (the diaphragm moving down), the fluid previously drawn in is forced out. Finally, the diaphragm moving up once again draws fluid into the chamber, completing the cycle. Diaphragm Pumps deliver a hermetic seal between the drive mechanism and the compression chamber, allowing the pump to transfer, compress, and evacuate the medium without a lubricant, which is preferred. Even though the sample won't be in contact with the pump, it is preferred to have a dry type of pump to avoid possible contaminations instead a wet one, where gas is exposed to water or oil during the pumping.[48][49][50][51]

	3014VD DC	**KNF NMP850KNDC**
Company	Thomas	KNF
Power supply	4.5V	12 V
Power consumption	840 mW	1.1 W

	3014VD DC	KNF NMP850KNDC
Working range	0.8 l/min	4.5 l/min
Maximum pressure	20 KPa	150 KPa
Dimensions (mm)	43.5x22.5x33.4	80.5 x 38 x 54
Weight	43 g	210 g
Price (EUR)	84.80	-

Table 4. Vacuum pumps comparison.

5.1.5. Electronics

To do so, Different designing software can be used for the obtention of a printed circuit board (PCB), as required as further explained in next chapter, each having their advantages and drawbacks. Three computer-aided design (CAD) software have been selected due to recommendations to ease the comparative study.

- DesignSpark PCB: it is a free electronic design automation software, specially designed for rapid prototyping and turning circuits into testable boards. DesignSpark PCB resulted from a collaboration between electronics distributor RS Components and EDA software developer 'Number One Systems'. It is one of the most most accessible and comprehensive options for entry level users.[52]

- Multisim + Ultiboard: Multisim integrates SPICE simulation with interactive schematic environment to visualize and analyze electronic circuit behavior. Once the design has been finalized, the circuit can be transferred to Ultiboard for PCB layout. Pricing of the license goes from 600 to 4500 €/year4 for Multisim and from 600 to 3500 €/year for Ultiboard [53][54]

- EAGLE: EAGLE is a scriptable electronic design automation (EDA) software with schematic capture, printed circuit board (PCB) layout, auto-router and computer-aided manufacturing (CAM) features.It was a development by CadSoft Computer GmbH, company was acquired by Autodesk Inc. in 2016. Since EAGLE version 8.0.0, there are Premium, Standard, Free, and Student & educator editions, with prices ranging from being free to 510$ a year.[55]

5.1.6. Vacuum chamber

The chamber in which the sample collecting container is placed performs its duty by allowing the creation of vacuum around the container, a sampling bag, and helps the sample get collected into it. It is accomplished thanks to the pump but without the sample going through it so all contamination is avoided.

In the first prototype a methacrylate box with three inputs was build. One is the valve output and sampling bag input, the second one the pipe through which the vacuum pump suctions the air inside the chamber to take it outside, and the third one was created for the placing of a manual valve to remove the vacuum. Inside this chamber, the container is placed and connected with the first input.

This methacrylate box created for the first prototype has one main inconvenient. The closure system consists in 8 screws that have to be tightly adjusted or removed each time the sampling bag has to be extracted from the sampler, making the procedure inconvenient and time consuming.

For this reason, alternative vacuum chambers are researched for a future final device.

Two interesting examples are the Vacuum Bag Sampler Model 1062 and the VC20 Vacuum Chamber Sampler. Easy to use, portable and cost-effective air sampling collection devices with a structure, that can be used as a reference in future vacuum chambers. Nevertheless, these models aren't suitable for this prototype as they are both designed for a 10 L or bigger sampling bag capacity, and therefore, its size, weight and dimensions are too big for this application. [56][57]

5.1.7. Disposables

- **Sample container**

Direct breath sampling onto pre-concentration materials is possible (Basanta et al. 2010) [58] but most of the studies in literature make use of storage. Apart from polymer bags, constituting the majority of breath collection containers, aluminium bags can be found. Bio-VOC™, a late expiratory breath sampler, breath collection apparatus, and glass vials are suitable alternatives used. For its usage in the medical field, the ideal container should be cost and user-friendly, durable, inert and isolated from the external environment, avoiding the possibility of sample contamination.

Tedlar bags are the container used for the prototype developed, as they were already available due to its previous use in the first prototype. Given the construction of the prototype, no other alternative container would be suitable other than using an aluminum or multilayer foil bag instead of the polymeric one. Hereunder both types of bags are compared.[59][60]

	Tedlar bags	**Multi-layer foil bags**
Composition	Polyvinyl fluoride (PVF) polymer resin	4-layer (gauge nylon outer layer, polyethylene, aluminium foil, polyethylene inner layer)
Max. Operating Temperature (oC) **Thickness**	82 (polypropylene valves) 204 (stainless steel valves) 2 mil	82 4 mil
Specific Gravity (g/ml)	1.70	1.09
Permeability (g/(m^2 x d))	9-57 for H20, 172 for CO2, 50 for O2	0.0078 for H20, 0.0078 for CO2, 0.0078 for O2

Advantages	Good resistance to UV light. Longer sample storage.	Sample stability for up to 5 days, including VOCs. Ideal for low molecular weight compounds
Limitations	Some VOC background for DMAC and phenols.	Not suitable for collecting low ppm VOCs.
Cost	Economical	Moderate cost

Table 5. Breath storing containers comparison.

- **Mouthpiece**

A mouthpiece acting as a disposable filter that prevents cross-infection is required. It will be necessary its substitution after each test, analogously as its done in other respiratory tests.

Vitalograph is the provider of the mouthpiece chosen that likewise other component selection, was already available I the laboratory.[61]

5.1.8. Case and tubing

To assemble all components in a sturdy robust structure, a 3D-printed case is designed and manufactured. Furthermore, it is seen that the previous prototype had a series of pieces attached to one another that could be replaced by a custom T- shaped piece.

It was also taken into account when designing the case and assembly of components, that the tubing system of the whole prototype is best when shortened as much as possible, so all along the the circuit length leakage is avoided, as well as a condensation in it.

Concerning the materials, PFTE was used for the connexion tubes forming the circuit that conducts the sample from the subject into the bag and the air from the chamber to the outside. It is characterized for being biocompatible, non-toxic and inert, just as required.

Secondly, the case material of choice was polylactide (PLA). It is one of the most common plastic filament materials for this manufacturing technology. It is a biocompatible, non-toxic and biodegradable polyester derived from naturally occurring organic acid thermoplastic.

For the accessory ambient air sampler, another case and tubing are employed made for the exact same materials.

In addition, CAD modelling software for the 3D pieces designing is studied.

- Fusion 360: it is a cloud-based computer-aided design (CAD/) and computer-aided manufacturing software by Autodesk that allows collaborative product development, enabling exploration and iteration on product ideas and collaboration within distributed product development team. Fusion 360 combines organic shapes modelling, mechanical design and manufacturing in one comprehensive package.Its license has a cost of 500$ a year or a free limited non commercial license.[62]

- TinkerCAD: is a free online collection of software tools for beginners to introduce themselves to modelling. It runs in a web browser, so no download is needed, and it is compatible with 3D printers, but it is quite limited.[63]

- Blender is a free and open-source 3D computer graphics software that can be used for creating animated films, visual effects, art, 3D printed models, motion graphics, interactive 3D applications, virtual reality, and computer games. Blender's features include 3D modeling, UV unwrapping, texturing, raster graphics editing, and rigging and skinning among many others. It's wide range of functions as well as its design goal being to provide speed for the designers by creating a large number of shortcuts, accessed by keystroke, difficult its learning curve.[64]

Fusion 360 is finally the software of choice, with the non-commercial license for its usage.

5.2. Software

The electronic components of the embedded system developed in the present project are fully controlled and commanded by a code programmed in an environment chosen according to the requirements of the device.

The choice made is LabVIEW and it is maintained from the first prototype, as it satisfies the characteristics needed, being the main one to create a user friendly interface for the acquisition of exhaled breath samples from a patient.

Laboratory Virtual Instrument Engineering Workbench (LabVIEW is a system-design platform and development environment for visual programming by National Instruments. It is used for applications that require test, measurement and control with rapid access to hardware and data insights.[65]

Although its license cost 400 $ a year, the University of Barcelona provides a free student version. Also, National Instruments provides a free non commercial use version called LabVIEW Community Edition.

As an Arduino board is used for the ambient air accessory sampler, the open-source Arduino Software is also used, as it facilitates to write code and upload it to the board. The environment is written in Java and based on Processing and other open-source software.

Furthermore, a proof-of-concept is carried out with a gas chromatography-thermal conductivity detector (GC-TCD) machine. Lastly, R and R Studio is used to analyze the results of this test.

5.3. Proof of concept

For the proof of concept, which will be later further detailed in Chapter 6, a Gas Chromatograph with a Thermal Conductivity sensor is used.

Chromatography is a laboratory technique for the separation of a mixture consisting of the dissolution of the mixture sample in a fluid called the mobile phase, which carries it through a system on which is fixed a material called the stationary phase. The different components of the sample have different affinities for the stationary

phase, so the different molecules stay different periods of time on it, depending on their interactions with its surface, causing them to separate.[66]

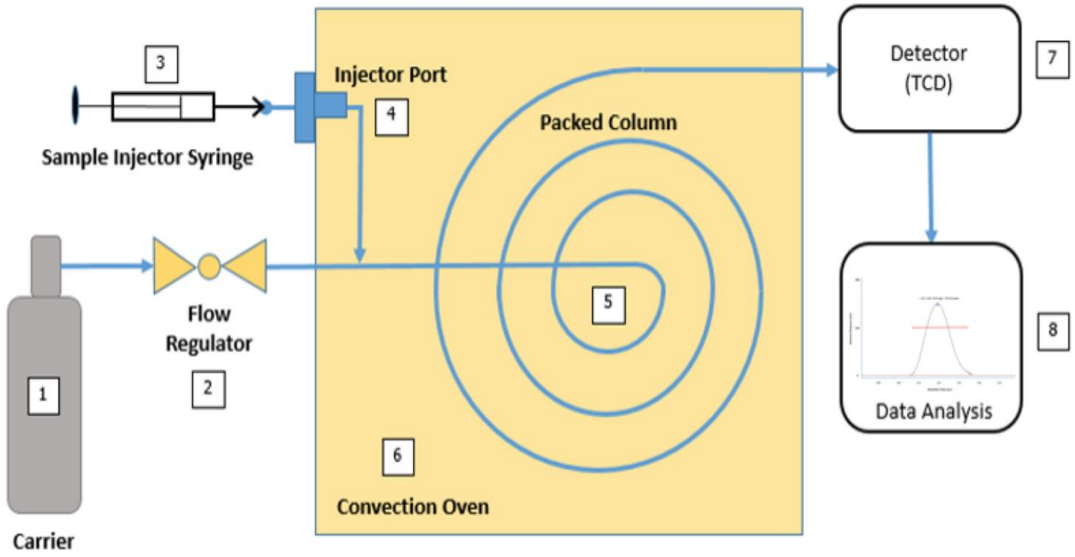

Figure 3. GC TCD schematic

Thermal conductivity detector (TCD) is a commonly used detector in gas chromatography that relies on the thermal conductivity of matter passing around a coil where a current pass through.

TCD works by having two parallel tubes both containing gas and electrically heated coils in a temperature-controlled cell.

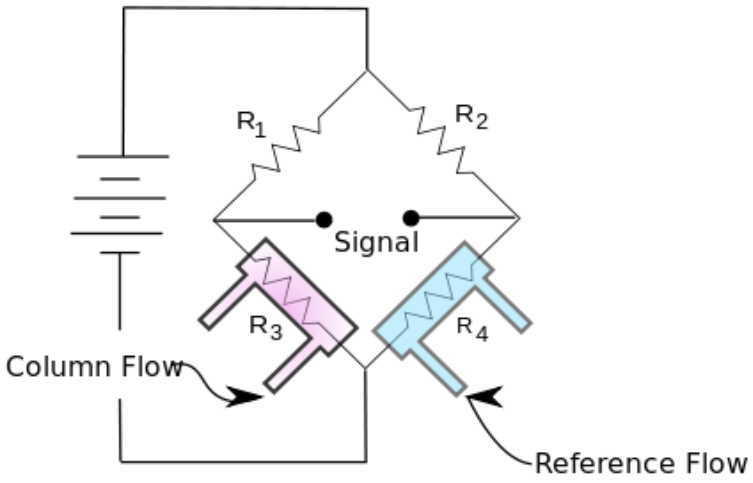

Figure 4. TCD Wheatstone bridge circuit

Helium or nitrogen, serve as the carrier gas (mobile phase) because of its relatively high thermal conductivity which keeps the filament cool and maintains uniform resistivity and electrical efficiency of the filament.

One tube holds a reference gas and the sample to be tested is passed through the other, and the changes in the thermal conductivity are compared to the reference flow of carrier gas.[67] [68][69]

When analyte molecules elute from the column, mixed with the carrier gas, the thermal conductivity decreases while the filament heats up and changes resistance. This resistance change is sensed by a Wheatstone bridge circuit which produces a voltage fluctuation, causing a detector's response, a signal to be produced. Sensitivity is proportional to the filament current and inversely proportional to the temperature around that detector as well as flow rate of the carrier gas.[66]

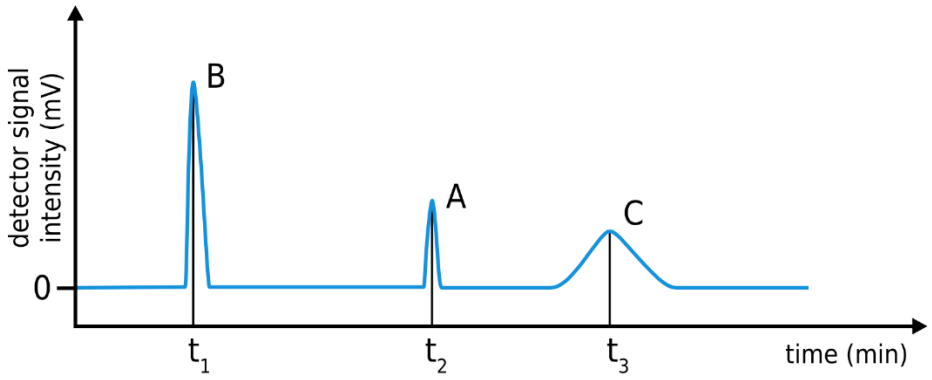

Figure 5. Reference of a GC TCD output signal

Therefore, when a compound passes through the TCD, the voltage signal changes, shaping a peak on a baseline. The peak position on the baseline reflects the compound type and the area represents the compound concentration.

A chromatogram is the output of a chromatographic run, a graphical representation of our separated eluents which can be used to identify compounds and to determine their relative concentrations within the mixture. [70]

6. Detail engineering stage

This chapter reflects the development process of the project, from designing the prototype to its assembly, specifying all chosen components and specifications.

The software implemented in order to make the hardware functional is also displayed, and flow diagrams or protocols showing how each user interface or code is used to control the device are attached in an annex.

Finally, the results from the proof of concept are shown in this section too.

6.1. Design bases and criteria

From the previously presented concept engineering analysis, different options are explored for hardware components as well as for software to be used to find the best alternative keeping into account the limitations and other considerations as it will be explained.

Concerning the components,most of them were already available, as they were used in the first prototype and therefore considered a suitable option, being this its selection criteria. This fact also helped keep the project

within budget, which is another consideration taken into account for the components taking into account that the goal is the development of a cost competitive breath sampler.

On the other hand, regarding the software a similar situation occurred. Code programmed with the objective of developing an user interface had already been implemented but not tested, and in its choice license costs and functionalities were already been considered and considered suitable. Therefore, the choice was upheld. As far as the CAD modelling software, the previously mentioned possibilities were researched and tested to finally choose the one that resulted the best fit.

Finally, the proof of concept design was strongly impacted by the current pandemic situation. Although it would have been interesting to be performed with a group of real patients, stablishing a comparationn with a control healthy group and carrying out an in depth data anlysis to check the robustness of the results that can be obtained thanks to the prototype, but because of bureaucratic issues and the COVID-19 situation it is out of scope. Therefore, the proof of concept is defined in order to check that the prototype works as intended and collects samples from exhaled breath in a standardize manner, so as to validate its functionality.[71]

6.2. Equipment and component selection

6.2.1. Breath sampler

Firstly, all employed hardware components are listed along their specifications.

- **Arduino UNO.**

An Arduino Uno and an ELEGOO UNO R3 microcontroller boards, both based on the ATmega328P are used for availability and suitability reasons in the project.

These boards can be powered via the USB connection or with an external power supply. The first option is the choice made in this case, because as it will be seen in following sections, the Arduino needs to be connected to the computer in order to send and receive data. [72]

	Arduino UNO
Microcontroller	ATMega328P
Operating voltage (V)	5)
Digital I/O pins (V)	6-20
Analog Input pins	14
DC current per I/0 pin (mA)	6
DC current FOR 3.3V pin (mA)	20
Flash memory	50
SRAM (KB)	2
EEPROM (KB)	1

Clock speed	16 MHz
Dimensions (mm)	68.6 x 53.4
Weight (g)	25

Table 6. Arduino UNO technical specifications.

Figure 6. Arduino UNO board.

- **PCB**

With the purpose of having all components connected between themselves and with the governing microcontroller at the same time that the easy exchange of components in case of malfunction or brokenness is allowed, an Arduino shield PCB (Printed Circuit Board) was designed with DesignSpark PCB. A previous preliminar design from the first prototype was used as a reference component wise, but some changes such as removing a push button for stopping the code or turn off the bomb and valve were made. Custom schematic symbols and PCB components were created In order to achieve the desired final results.

Due to a necessary hardware redesign, consisting in the implementation of a second solenoid electrovalve before the pump and a pump exchange, a second version of the shield PCB had to be made. This change implied the necessity to use an external power source to power these components and the implementation of extra connectors, an additional transistor, and an overall electrical circuit new design.

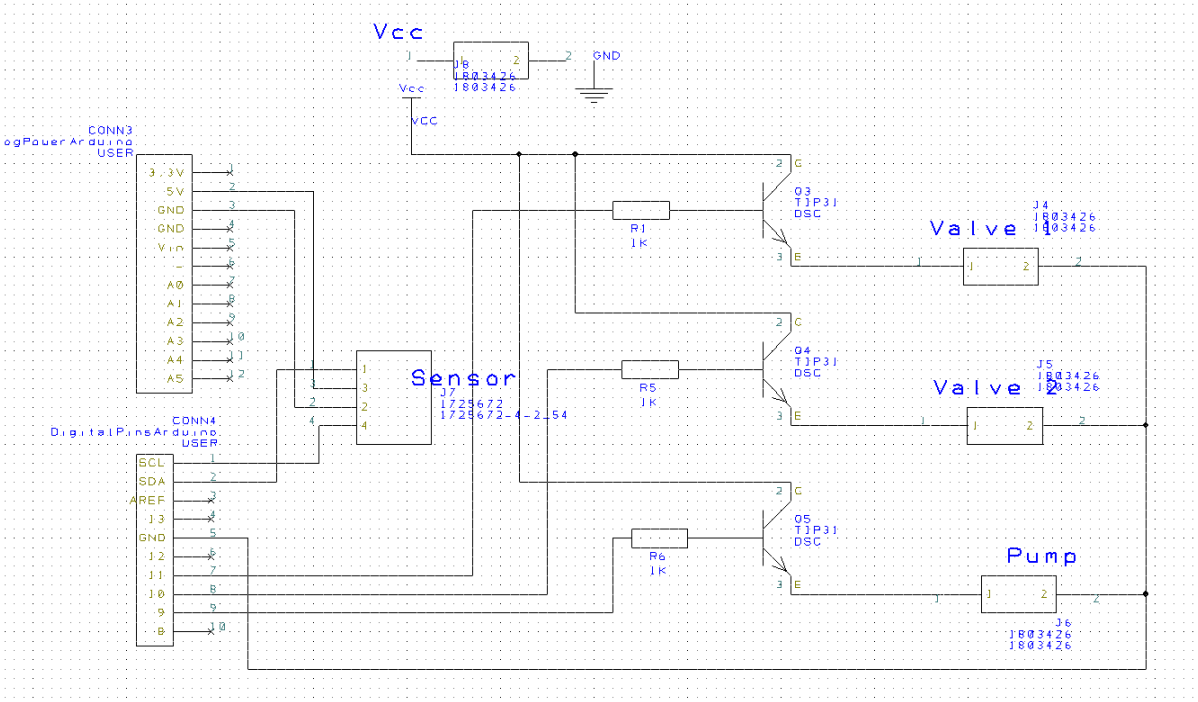

Figure 7. Design Spark PCB Schematic.

The final schematic has been designed so that the flow sensor has a custom connector through which it sends data and is directly powered by the 5V output voltage from the Arduino. The sensor is therefore connected to SDA, SCL, GND and Vcc following its datasheet.

Then, the other components (the pump as well as the two valves) are powered by the external source, and this process is governed by the Arduino and regulated thanks to three switch-configurated transistors that allow the flow of the power when a signal from the microcontroller is received.

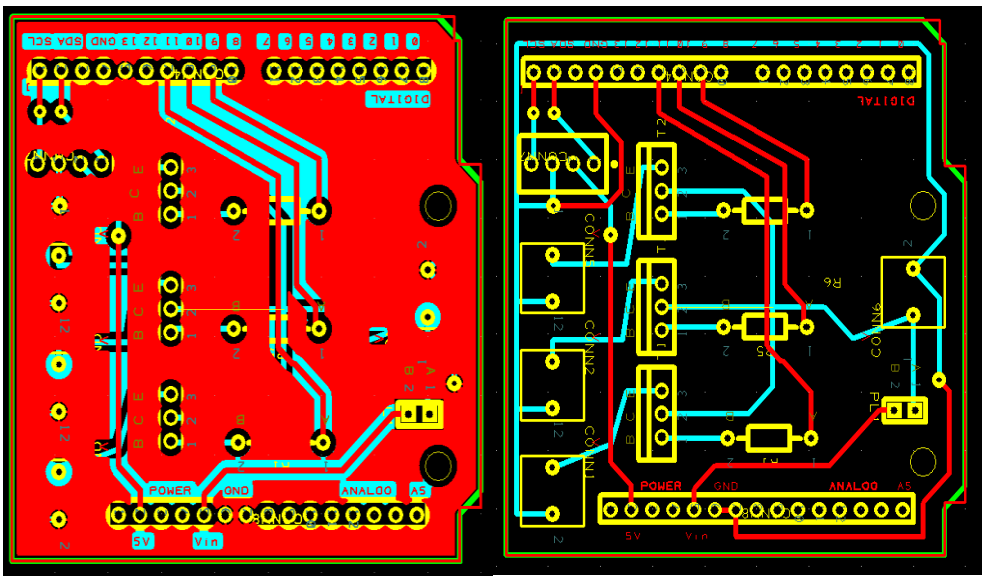

Figure 8. PCB layout of the breath sampler's electronics with (left) and without (right) copper pour.

As seen in Figure 8, the PCB is designed to be a shield to mount on top of the Arduino board to give extra functionality to the Arduino. It communicates with it through all the pins, even though just a few of them are used for this application.

It is designed so power goes into the PCB on one end of it, and the valves and pump are connected to the other in an organic way that allows a neat distribution of all components. All of them are connected through traces places in two layers so no crossings happen among different ones, and therefore a double-sided PCB is created.

It was manufactured in the Electronics Department of the Physics Faculty workshop and soldered by the student with supervision from the tutor, and no solder mask or silkscreen were added, so the name of the pins and connectors' labels were drawn directly on the top copper layer for reference.

- **Vacuum Chamber**

A methacrylate chamber existing from the first prototype is utilized in the context of this project as well, with 170x150x150 mm dimensions.

It consists of a transparent tetrahedral box originally with three inputs, one for the electrovalve output (where the sample will enter the bag), one for the vacuum pump and another for the manual valve (to remove the vacuum). However, the third one was closed and the manual valve removed. The reason for it being that due to the location of all inputs in the same box face, a gate in the case would be needed to operate it (as done in the first case which will be explained later), but the best solution for it would be to include the functionality of removing the vacuum in the user interface, avoiding any possible leaks from having the extra input this way too.

As leaking was a strong problematic when trying to operate with the prototype, not being able to achieve a substantial vacuum, and more importantly, maintain it, a series of test were carried out, including:

- Visual inspection of balloons' volume inside the chamber: it was expected to see it expand as vacuum was created when the air inside the chamber was removed, but initially with the first pump no difference was seen. When it was substituted by the final pump of choice, the balloon expanded, but came back to the original volume after a few seconds.

- Manual valve sound verification: if vacuum were achieved, when opening the valve for releasing the vacuum, a flushing sound would be made, that was not achieved initially either. Then, it was achieved but only if performed right after stopping the pumping process, if done later, no sound was made, which indicated leaking.

- Vacuum chamber immersion: it was checked that once the pump was updated and the manual valve showed that vacuum was created but not maintained, leaking existed. For this inspection the box was immersed into water and air bubbles were seen in the surface, indicating the escape of air from inside the chamber.

With these results in mind, corrective measures were taken. The first one was sealing with a specific silicone sealing all edges of the chamber. Secondly, the rubber joint in the lid was replaced with a thicker alternative of the same material, and finally, a hardware redesign was made, consisting in the implementation of a second solenoid valve into the system in the second input hole before the pump in order to maintain the vacuum after the pump stops working, help seal leakages and avoid the return of extracted air; and an exchange of the valve, incorporating a 4.5 L/min extraction flow one instead of the 0.8 L/min present before to be able to extract a bigger volume of air and increase the vacuum achieved inside the chamber.

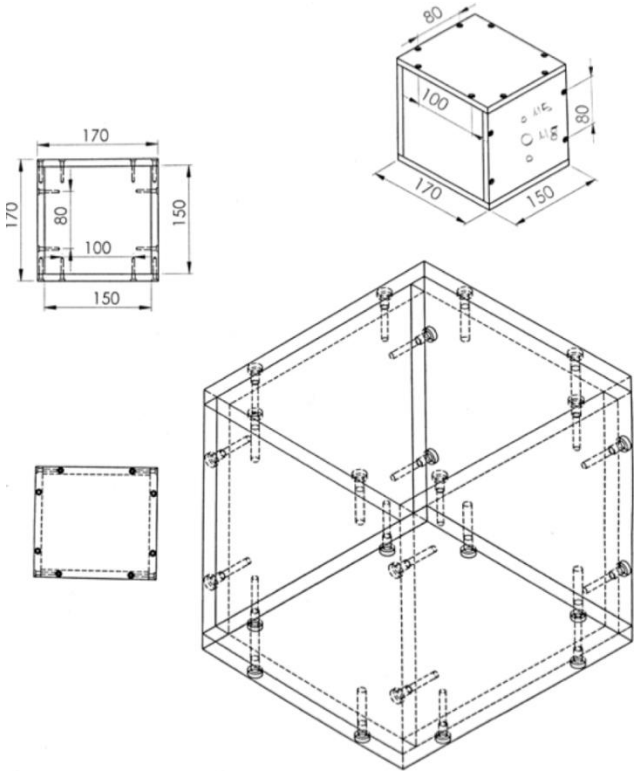

Figure 9. Vacuum chamber dimensioned construction layout

- **Tedlar Bags**

A container for the collected exhaled breath sampler storing prior to its analysis is required. For this,Tedlar sample bags are used. They are the choice made due to its availability at the laboratory, and because they have the appropiate features for this particular application.

There is a series of requirements that the container has to meet, being the main one not reacting with or alter the composition of a wide range of collected chemicals to assure sample integrity, and it is satisfied.

For its use in the breath sampler prototype, 0.5 litres sized bags were used, although a wide range of sizes are available. Another feature that has to be selected is the closing valve, which can either be single or dual, made from polypropilene or stainless-steel. Single polypropylene valve type is chosen as it is a good fit for the application operational and cost wise. Its septum allows the introduction of a gastight syringe to extract and concentrate the sample and analyse it. [59]

Figure 10. Tedlar bag from Restek.

- **Mouthpiece**

Following the first prototype, a mouthpiecefrom Vitalograph is used to ease the performance of breathing into the device for the patient, to provide protection again cross-infection and act as a disposable filter. [61]

Figure 11. Mouthpiece from Vitalograph

- **Non-rebreathing valve**

The two-way non-rebreathing valve is used to ease spontaneous breathing and control the flow using diaphragms. It has three ports identified as Inhalation, Exhalation and Mouth port. Its materials are also biocompatible and don't interact with the sample. [73]

Figure 12. Two-way non-rebreathing valve from Hans Rudolph inc.

- **Tubes**

In order to achieve a continuous circuit for the gas to flow through, the components of the prototype need to be attached and connected with tubes, to conform a tube with different stages where the sample will flow through along the prototype.

A first tube connects the mouthpiece with the flow sensor ,that is at the same time connected to a two-way non-rebreathing valve by another tube. Then, the exhalation port of the non-rebreathing valve before the gate consisting in a solenoid valve that allows or not the air to go through, a T-shaped tubing piece is needed and designed to be manufactured with a 3D printer. It has one input where the air flow enters, and two outputs, one that leads to a tube connected to the valve to take air as sample towards the container to be stored, and another one as an exit for the air that is not of interest as a sample.

All tubes are carefully measured so there is no need to glue them and may allow its exchange as well as the exchange of the valves and sensor if needed, but still be part of a solid construction.

- **Case**

A case is designed using Fusion 360 to assemble the whole of components into a structure and make the device sturdy and robust.

A sliding lid is incorporated to facilitate the case opening process and holes for the device connexion to the current and the Arduino to the computer as well as for the mounting of components are made.

It must be mentioned that a first version was made for the prototype assembly before the redesign in hardware featuring a gate to open the manual valve that was removed when this function was included in the software interface. A redimension of it was required too, as it no longer fitted the original components, and it was also improved to place them securely and fix them so the distances of the tubing system would be the shortest possible, avoiding condensation in the walls of the tubes.

It must be mentioned that at the time of the writing of this memory, due to the time required for printing the case and the waiting list due to demand at the department's workshop , this second case is still in manufacturing phase. It is expected to perform the final assembly with it nearby the presentation date.

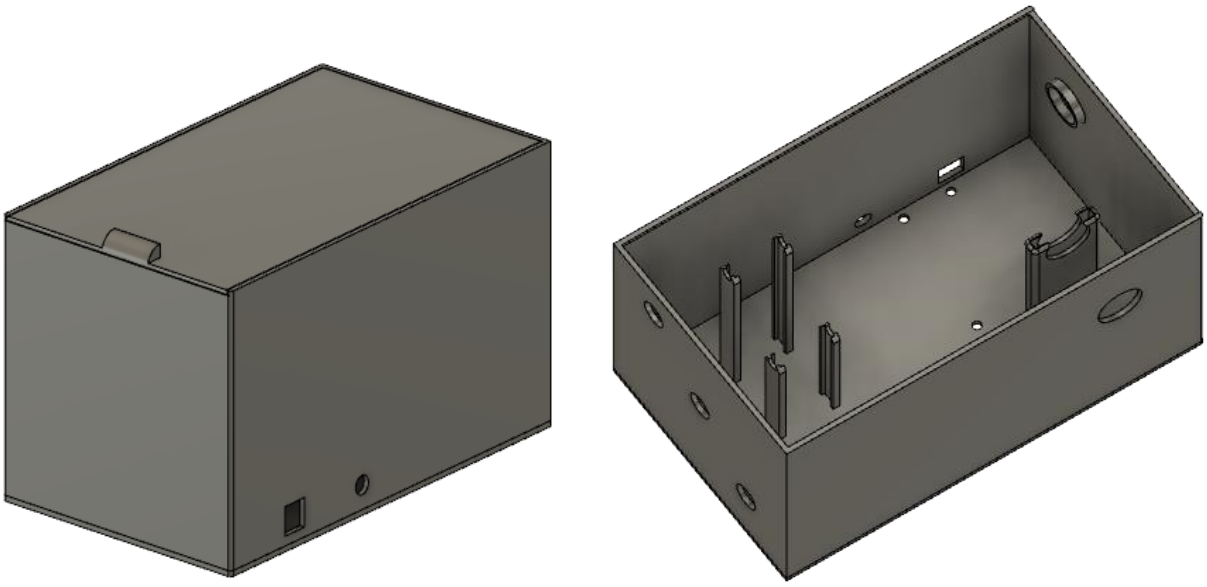

Figure 13. Breath Sampler case design in Fusion360.

-KNF NMP850KNDC Pump

The 3014VD DCvalve of the first prototype as mentioned in the Vacuum chamber point of this section, had a small extraction capacity, so it was replaced with another diafragm gas sampling pump from KNF [50] available in the lab.

	KNF NMP850KNDC
Company	KNF
Power supply	12 V
Power consumption	1.1 W
Working range	4.5 l/min
Maximum pressure	150 KPa
Dimensions (mm)	80.5 x 38 x 54
Weight	210 g
Price (EUR)	-

Table 7. Pumps technical specifications.

Figure 14. KNF NMP850KNDC pump technical specifications.

- **EV-2-12 Valves**

The valves were also part of the hardware redesign applied to the prototype, incorporating an extra one as explained before. Because of their discontinuity by the provider, a second identical valve to the one present before could not be acquired, and therefore, a 12V version of it was the choice to be implemented. Its specifications are listed in Table 8. [74]

The second valve is implemented in order to seal the chamber when the pump is not extracting air from it and keeps the generated vacuum avoiding a rise in pressure in the chamber each time the pump stops functioning.

	EV-2-12
Connection	18" Wire Leads Side (Radial)
Cycles	Over 1 Billion
Function	2-Way Normally-Closed
Material	Nickel Plated Brass Body & Core
Medium	Clean, Dry Air (40 micron filter)
Mount	Standard In-Line Mount
Mounting	#6-32 thd. Mounting Holes
Ports	#10-32 Female In-Line Ports
Power Consumption (W)	0.67
Pressure (Flow) Range	105 psig (17 l/min@100 psig)
Response Time	5 to 10 milliseconds (nominal) @ 25 psig
Seals	Nitrile
Series	Standard
Unit	Imperial
Voltage	12 VDC
Weight	0.1700
Wire Size	26 Gauge
Company	Clippard

Table 8. EV-2-12 technical specifications.

Figure 15. EV-2-12 valve

- **Power supply**

The hardware redesign needed for the prototype to function as intended resulted in the necessity to implement an external power supply of 12 V to fuel the valves as well as the pump, that required a 12V power source instead of the 5V that hardware required before, that could be given from the computer through the microcontroller board. Its specifications are displayed in Table 9. [75]

	25W Single Output Switching Power Supply
DC Voltage (V)	12
Rated Current (A)	2.1
Current Range (A)	0-2.1
Rated Power (W)	25.2
Efficiency(Typ.)	9-57 for H20, 172 for CO2, 50 for O2
Input Voltage Range	88 ~ 264VAC 125 ~ 373VDC (Withstand 300VAC surge for 5sec. Without damage)
Dimension (mm)	78*51*28
Provider	RS Components

Table 9. 12V Power source technical specifications.

Figure 16. 12V Power source by RS.

6.2.2. Ambient air sampler

- **ELEGOO UNO R3**

The microcontroller board used for this device, unlike the breath sampler, is powered by a powerbank, as there is no data exchange requirment, and it is a more ecologycal and reusable choice compared to single use batteries that still keeps the device portable.[76]

- **Power Bank**

A Mi Power Bank 3 10000mAh 18W Fast Charge is chosen as power source for availability reasons to power the microcontroller. [77]

	Mi Power Bank 3
Battery Type	Lithium polymer battery
Cell Capacity	37 Wh 3.7 V (10000 mAh)
Rated Capacity	5500 mAh (5.1 V/2.6 A)
Input Port	Micro-USB/USB-C
Output Port	USB-A
Dimensions	147.8 × 73.9 × 15.3 mm
Charging Time	Approx. 4 hours (with the

Table 10. Mi Power Bank 3 technical specifications.

Figure 17. Mi Power Bank 3.

- 3014VD DC pump

The 3014VD DC pump, with part number 30140004 specifically, is chosen as its supply voltage is 4.5 V and doesn't require an extra battery to operate, so it can be connected to the Arduino output voltage pin. Moreover it is small and portable. [51]

This component was already in use by the first breath sampler prototype, but as it was proved when tested, it fell short attempting to create vacuum in the chamber, and it was replaced. Nevertheless, it is suitable for creating a suction force that allows the collection of samples from the ambient air, and was used for that purpose.

	3014VD DC pump
Max Flow (l/min)	0.8
Motor type	Ironless core DC
Voltage Supply (V)	4.5
Power Consumption (mW)	840
Max. pressure (kPa)	20
Weight (g)	43

Table 11. 3014VD DC Pump technical specifications.

Figure 18. 3014VD DC Pump by Thomas.

- Jumper cables, switches and leds

Two leds were implemented as a visual indicator of the sampler's being into action, and two switches as well in order to select the bag size and action the device. Generic Arduino cables were used for the connection of the components too.

-Tedlar bag
A Tedlar bag identical to the one used for the Breath Sampler was used to store the ambient air samples.

-Tubes
PFTE tubes were used here too for the input and output airways of the pump.

-Case
This second case is designed using Fusion 360 as well to assemble the components in a compact portable way, and a sliding lid was the closing of choice again, as components remained easily accessible this way but still protected. Pictures of the final device's assembly can be found in the annexes.

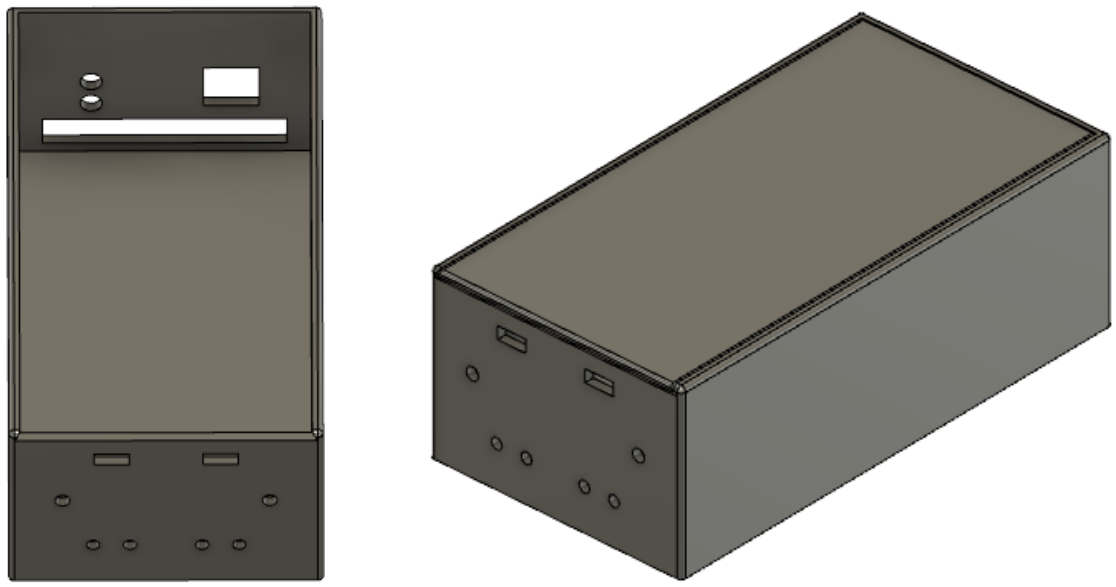

Figure 19. Ambient air sampler case design in Fusion 360.

6.3. Software

The developed code for both devices, the breath sampler as well as the ambient air sampler are displayed and explained hereunder in this chapter.

6.3.1. Breath Sampler

The breath sampler is controlled through an user interface developed in LabView, an environment that allows an easy control and interaction of hardware through design engineered user interfaces, implemented by using code block diagrams.

Due to the usage of an Arduino as a microcontroller, an interface with this embedded platform called LINX is used to pass on the LabVIEW code and operate the Arduino with it. It is provided as an open source by Digilent.

LINX includes also VIs for over 30 of the most common embedded sensors as well as hardware agnostic APIs for accessing peripherals like digital I/O, PWM, I2C, SPI and UART. [78]

In this project, APIs for accessing digital I/O pins and for I2C peripheral, as it is the flow sensor, are used.

A main interface menu is created where the user can select the action to be executed, or in other words, the VI to execute. The first one is the breath sampling mode, and the second one a vacuum releasing mode that functions by opening the solenoid valve connected to the pump as it was manually done after a test with the previous prototype.

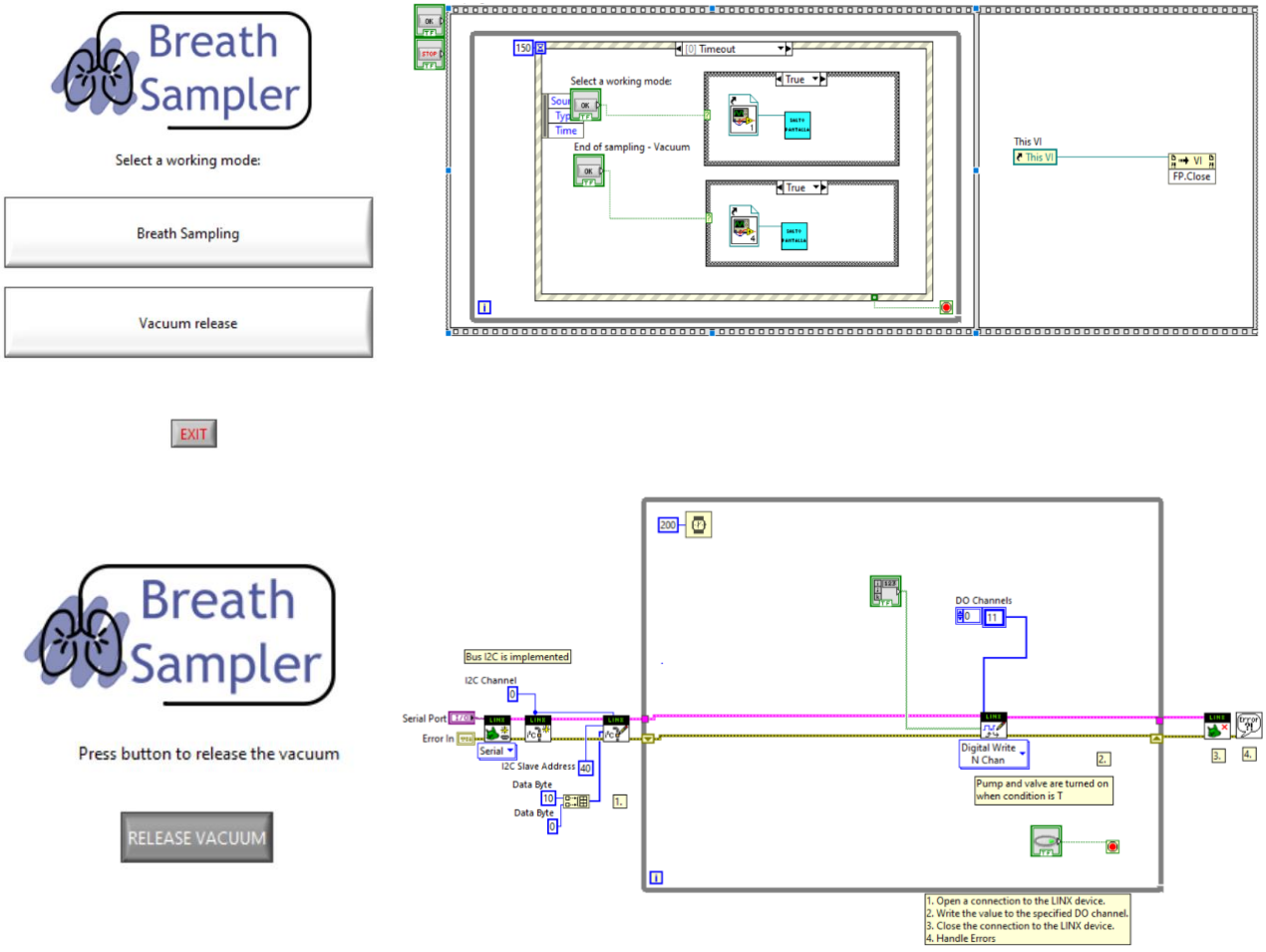

Figure 20. Labview UI main menu (up),Vacuum release mode UI (down) and their corresponding block schematics.

The main VI, for the breath sampling, consists in a block diagram configured to start the interaction with the Arduino and start the bus I2C. The address of the sensor is sent to start the connection with the right slave and three bytes are read. From these bytes, the MSB (most significant byte) and the LSB (least significant byte) are used to calculate the flow using Eq. (1).

$$measured\ value = MSB * 256 + LSB \quad (1)$$

The flow of the respiration is then plotted in a Waveform Chart and used to turn on the pump and the valves simoultaneously so the sample enters the bag through the first opened valve, the pump extracts air to create the vacuum that helps to do it, and the second valve opens the pipe connecting the pump allowing the extraction.

Initially and based on the first prototype, the device would be put into action when the flow is positive and the difference between flows are smaller than -5, but a case structure was implemented to be able to select the condition, based on the consideration that on diseased patients with respiratory difficulties this criteria might not achieve the actioning of the components the sufficient time to collect the sample or might not even trigger them at all.The -5 value was kept, as it was the value extracted from the first prototype approach searching for parameters to obtain just the exhaled portion of air that is in contact with the alveoli, but two more option were added, one of them that would facilitate the collection of almost all exhaled air, and an intermediate extra one for subjects with breathing difficulties but to whom the breath portion selection could be applied and still collect sample.

A stop button is also implemented in the code to facilitate the stop of the experiment using the User Interface, which was previously done with a switch button in the PCB, and all values of time and flow can be saved in an Excel file for posterior study.

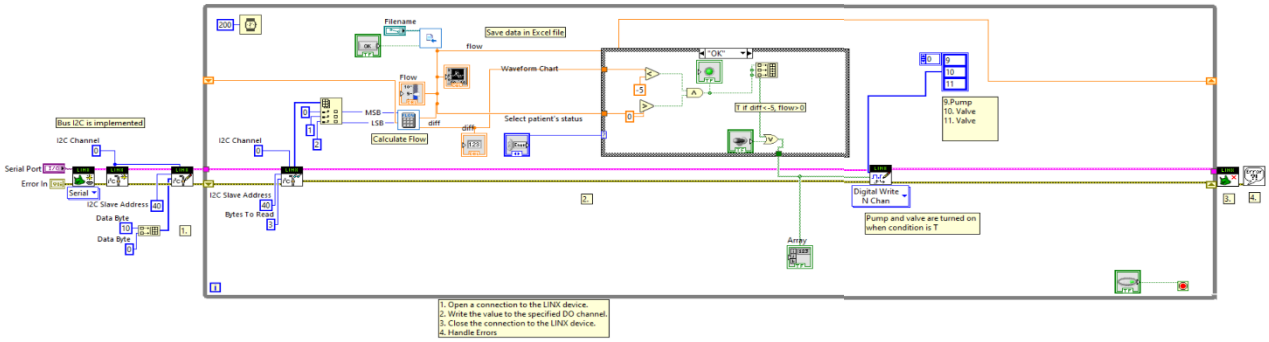

Figure 21. Breath Sampler VI block diagram.

The LabVIEW's block diagram is then showed in Figure 21 to have a visual explanation of the code details. Different SubVIs are used in order to write modules of code and sort the main VI. These SubVI can be seen in the annexes document. Zoomed versions of the figures in this are provided there too.

From the block diagram, a User Interface is arranged for the sampling mode where the flow can be visualized, the port of the Arduino Board configured, the program stopped, the valve turned on/off and see whether if they are on or off and save the data in a file.

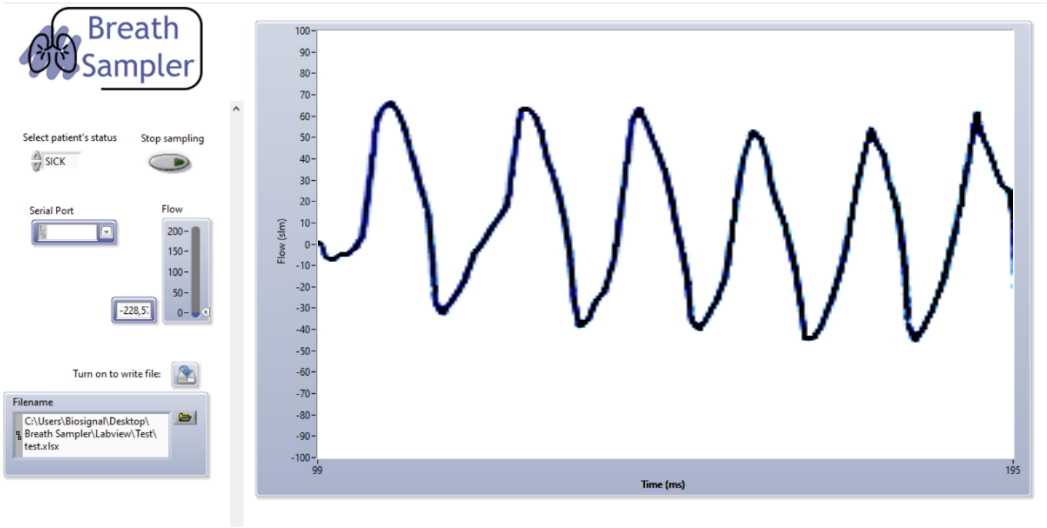

Figure 22. Breath Sampler sampling mode UI.

6.3.2. Ambient air sampler

To program the second microcontroller and achieve its functionality of sampling the air in the environment of the test being carried out with the breath sampler, Arduino IDE, is used.

The Arduino Integrated Development Environment (IDE) is the official open source software that is mainly used for writing and compiling the code into the Arduino Module, making code writing, compilation and uploading easy.

The developed code is written so the device can be autonomous. It is used to govern two buttons, each of them associated to a Tedlar bag sampler container size, so the pump is actionated when pushing any of them for an adecquate period of time according to the bag selected.

After this period of time is passed, the pump is switched off and the sampling bag can be disconnected from the device, and the device remains waiting for a push in the buttons to be switched on again. The corresponding Arduino IDE code is commented and displayed in the annex.

6.4. Flow diagram of principal and auxiliary processes

A flow diagram of the code developed for the user interface in LabVIEW and Arduino is created and shown to sum up and visualize how the data is treated and how the user interacts using the software.

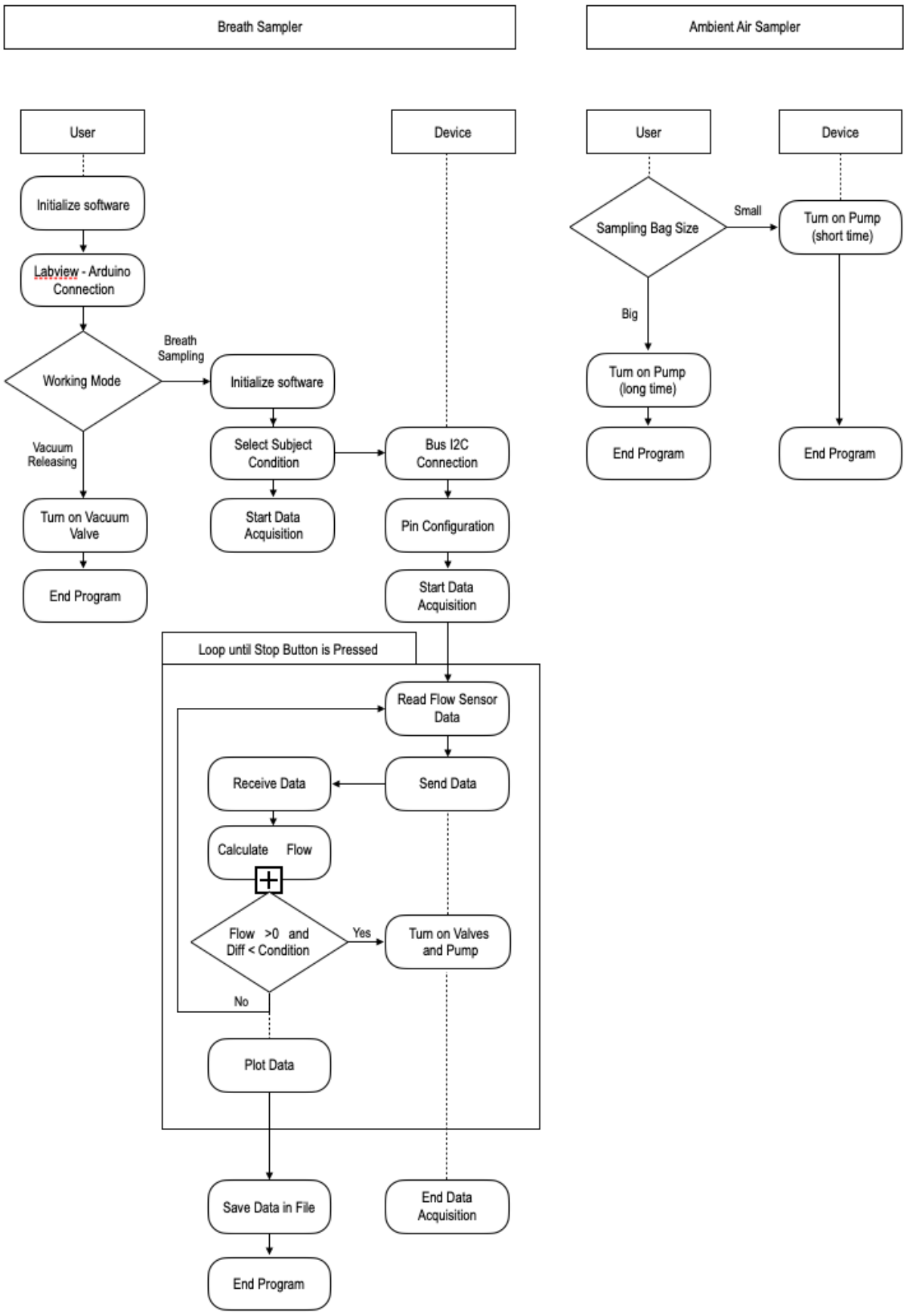

Figure 23. Flow diagram of principal and auxiliary processes.

6.5. Proof of concept

The purpose of the Proof of Concept is to demonstrate that the breath sampler device prototype performs as intended and collects exhaled breath samples. Its realization shows the device feasibility, verifying that its operational principle has practical potential.

Evidence derives from an experiment consisting of:

- Performance of a breath sampling procedure of a subject simulating real conditions

- Performance of simulated breathing with the prototype fed by a gas cylinder of known composition.

Prior to the performance of the experiment itself, a testing of the different conditions of sampling stablished was carried out. A subject, in this case the student developing the prototype, performed a series of breathing sets, inspirating and exhalating into the mouthpiece of the prototype.

It was checked, although qualitatively, that when performing breaths of the same length, depending on the condition chosen, the pump and valves were triggered sooner or later, discriminating more or less the fraction of exhaled air being collected.

Then, the experiment test were carried out. A breath sampling procedure of the student simulating real conditions of a patient diagnosis was conducted. The aim was to collect the sample and then analyse them in the GC-TCD available in the Instrumentation Laboratory of the Electronics Department. The procedure of breathing into the prototype was carried out for two minutes approximately. Initially, the Tedlar bag where the storage of the samples was done, was washed with argon gas and it was vacuumed so it was completely empty. A t the end of the procedure, it was noticeable enough that it had been filled along the process.

The bag was extracted from the vacuum chamber and with a gas sampling syringe with push-button valve lock, 1 microliter was extracted and injected into the GC-TCD.

The output chromatogram is a two-dimensional plot with the ordinate axis giving concentration in terms of the detector response, and the abscissa represents the time. The detector gives a response as a peak whose height is dependent on the concentration of the particular component.

Each peak represents a component present in the sample. Retention time is time interval between sample injection and the maximum of the peak. It is characteristic of the identity of the component under the operating conditions.

Identity of the components can be confirmed by making injections of reference material under the same operational conditions. In this case, air was injected as it can be seen in the figure below, to confirm the presence of oxygen and nitrogen and their retention time for referencing them into the sample analysis.

The result of the chromatogram run of the reference sample was the left graph of Figure 24.

It can be seen that there are two peaks corresponding to two different compounds detected shortly after the minute 2 of the beginning of the analysis , oxygen and nitrogen, whose height and therefore concentration corresponds to the one found in the atmosphere as previously seen in Figure 2. Documentation from the column manufacturer was consulted too to check and confirm the identification of the compounds.

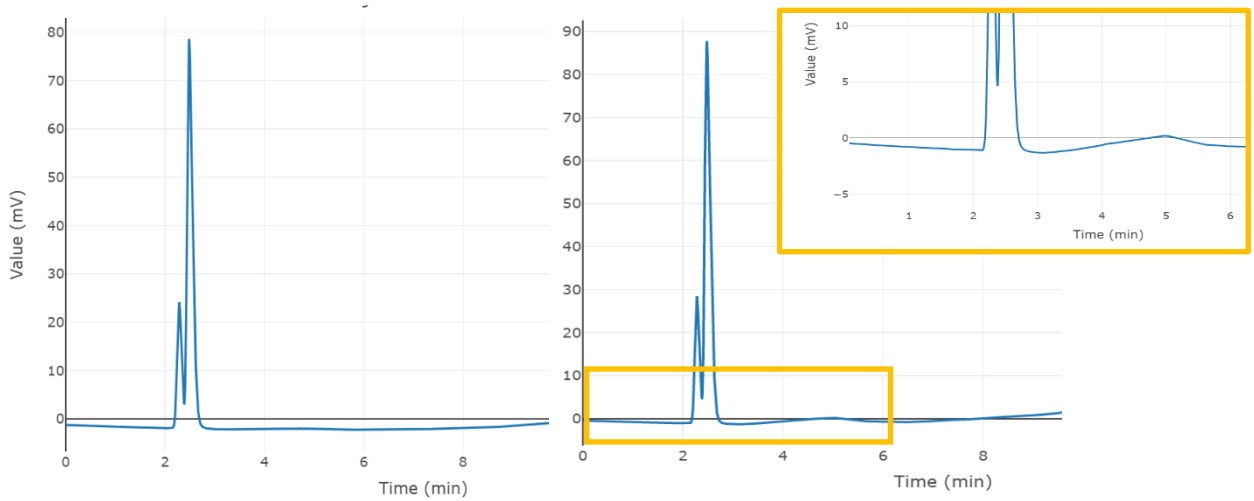

Figure 24. Reference (left) and breath sampling procedure (right) samples GC TCD chromatogram.

The result from the breath sampling procedure, reflected on the right graph in the Figure above, shows in addition to the two peaks detected in the air reference sample, a third peak around minute 5, which can be identified as CO2, metabolic product of the respiratory process present in exhaled air. It was consequently demonstrated that the air inside the Tedlar storing bag collected could be identified as exhaled air from the subject conducting the test, verifying the intended performance of the prototype.

However, due to the low concentration of CO2, the result was weakly perceptible, as seen in the zoomed window of the figure, and another test was performed.

Because of the column present in the GC, designed for light hydrocarbon and permanent gas mixtures separation, no additional component present in breath could be detected. [79]

To try and acquire a detection that clearly showed detection peaks from the prototype sampling collection , respiration was simulated by opening and closing a valve connected between the breath sampler input tube and a gas cylinder containing a 20% of methane and CO_2 as well as a 5% of hydrogen being the rest nitrogen. Once again, the bag was vacuumed before the procedure, and the extracted volume to be injected in the GC was 1 microliter.

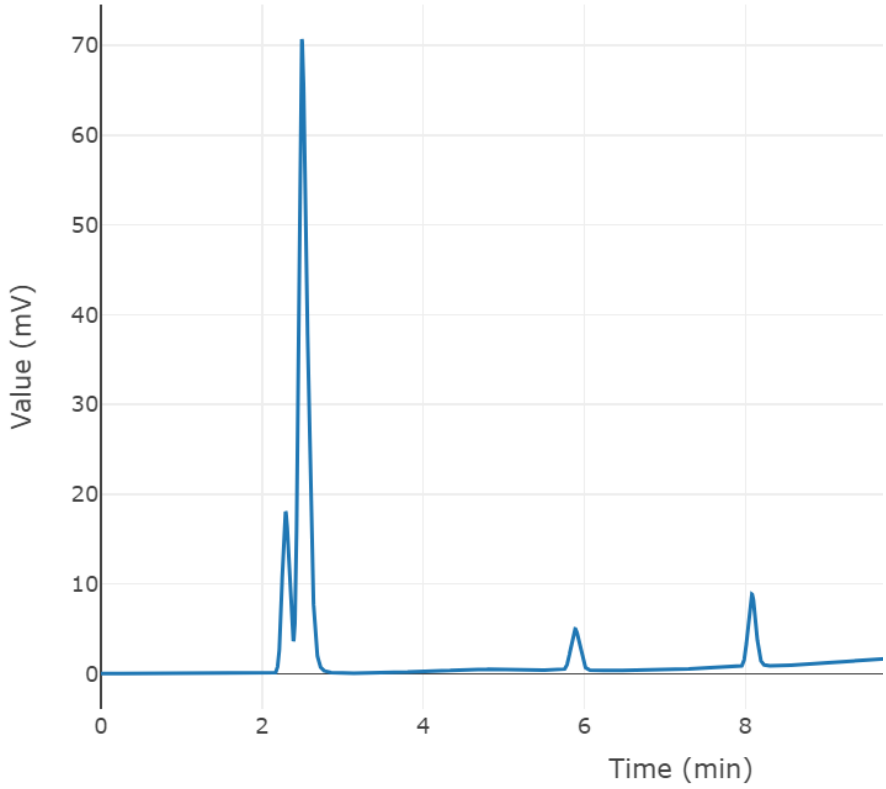

Figure 25. Simulated breathing with gas mixture GC TCD chromatogram.

The graph resulting from the chromatography run shows the peaks detected for nitrogen in minute 2:30, CO_2 near minute 5, and methane shorly after minute 8. A peak for oxygen is present too, which can be atributed to sample contamination in the process of extracting the sample from storage and injecting it in the GC, and the fact that the syringe valve may produce leaks. Overall, it can be concluded that the detected compounds corresponded to the ones expected to be found, as they were the ones present in the gas mixture introduced with the simulated breathing, reassuring the correct and expected breath sampling performance of the prototype.

All results were extracted from the output text data files from Chromeleon, the Chromatography Data System (CDS) Software used to run Thermo Fisher Scientific Gas Chromatograph in which the samples were analysed, and processed using R. Code of the plotting are found in the annex.

In addition to the detection data acquired reflected in the output file of the chromatogram, there is also other data such as:

- Filename and location of raw data generated during the run
- Injection information (Injection number, time, date, volume, dilution factor, weight)
- Chromatogram data information (Data points, detector, generating and exporting data system, signal unit, steps)
- Name of analytical method used

For the future development of the device, a different column or analytic method could be used so more compounds could be detected, and experiments comparing group of smoker or EPOC subjects and a control

healthy group would be interesting too. In addition, there is room for the deepening in chromatographic signal data processing comprising baseline correction, smoothing, peak alignment, and peak detection algorithms.

7. Technical feasibility

For the purpose of assessing the technical feasibility of the device developed, it is useful to study the technical specifications of the final prototype and identify and analyze its strengths, weaknesses, opportunities, and threats so favorable factors are focused on, and strategies to improve the unfavorable ones can be designed.

7.1. Technical specifications

The World Health Organization (WHO) provides guidelines around technical specifications for the procurement and acquisition process of medical devices as well as for the implementation, functioning and decommissioning of them.[80] From the Format of WHO technical specifications, the following table is created:

NAME, CATEGORY AND CODING		
1	Generic Name	Breath Sampler
2	Specific type or variation	Cost-competitive
3	Keywords	Breath, Sampling, medical device, non-invasive
PURPOSE OF USE		
4	Clinical or other purpose	Standardize the collection of breath samples, aiming for the isolation of air in contact with the alveoli
5	Level of use	Laboratory
6	Clinical Department	Clinical diagnosis
7	Overview of functional requirements	Obtains the flow of the inhaled and exhaled breath from the patient, keeps just the part that fulfills specific conditions, saves data
TECHNICAL CHARACTERISTICS		
8	Detailed requirements	Obtention sample within a Tedlar bag and patient data in an Excel file.
9	Display parameters	Display flow and difference between flow data acquisition with a continuous waveform display
10	User adjustable settings	Conditions applied to get the portion of sample
PHYSICAL CHARACTERISTICS		

11	Components	SFM3000 flow sensor, EV-2-6V valves, KNF NMP850KNDC pump, 12 V power supply
12	Dimensions	41,5 x 15 x 15
ACCESSORIES, CONSUMABLES, SPARE PARTS, OTHER COMPONENTS		
13	Accessories	Computer with USB port
14	Disposables	Tedlar Bag and Mouthpiece for each sampling procedure
TRAINING, INSTALLATION AND UTILISATION		
15	Pre-installation requirements	Labview, LINX extension for LabView
16	Training of user/s	Basic
SAFETY AND STANDARDS		
17	Risk Classification	II a
18	Regulatory Approval/ Certification	CE mark (EU), FDA approval (USA)
19	International Standards	ISO 13485, IEC 60601

Table 12. Breath Sampler technical specifications according to WHO guidelines.

7.2. SWOT analysis

To study the technical viability of the device, all factors with an impact in the device are identified and grouped into two different categories, internal and external or environment-related factors.

	HELPFUL	HARMFUL
INTERNAL ORIGIN	**Strengths** Reliable and reproducible collection of exhaled breath samples Adaptable to patient pathology or absence of it Non invasive Cost-competitive Easy to use	**Weaknesses** Vacuum chamber closure Limited proof of concept Biomarkers approved for breath analysis
EXTERNAL ORIGIN	**Opportunities** Growing interest in breath analysis, ongoing research Possibility of future collaboration with Hospital Clinics for studies	**Threats** Multiple companies with great budget allocation capacity and multidisciplinar researcher's knowledge developing similar devices

Table 13. SWOT analysis.

7.3. Study of the SWOT

The previous matrix reflects the identified factors according to its helpfulness or harmfulness and its origin, intrinsic to the device or belonging to its environment.

It can be extracted as a conclusion that although some weaknesses exist the one referring to the chamber's closure can be turned into a future progress and improvements, and the rest can be overcome thanks to the opportunities.

This can also be extrapolated to the strengths and threats pair of parameters, e.g. large companies with investment capacity are developing already similar devices nevertheless, the fact that the device developed in this project is low-cost can put the device as a big competitor in the market.

A SWOT analysis is a valuable analysis framework that can be used to easily get a sense of the feasibility of a project. It allows to identify the strategy to follow focusing and highlighting the strengths, being the main one in this case the low cost of the device, as well as getting the direction to keep moving and plan improvements with the weaknesses identified thanks to focusing and taking advantage of the opportunities. Also, it reflects about why makes the device less competitive than the ones in the market, for which the opportunities can be looked into too in order to solve them.

7.4. Availability and maintenance

It must be taken into account when assessing the technical feasibility the ease of acquiring all the components and technologies required to build the prototype. All components are currently commercialized and available or can be created (eg. The 3D printed casing or tubing). During the execution of the project, when the necessity of a hardware redesign arose, it was not possible to purchase a second identical solenoid valve as it had been discontinued by the manufacturer. Nevertheless, a similar version of it but with a higher power supply requirement was available by the same supplier. Although this was not expected, it did not supposed any limitation or inconvenience, given that the power supply had already been required to be higher to be able to supply the new pump.

As in terms of maintenance, the prototypes not very demanding. It does not require any calibration, so maintenance only includes change of the disposable components (mouthpiece and Tedlar bag) between each experiment, and tubes or valves need to be cleaned periodically.

7.5. Safety and security

When developing a medical device, it has to undergo certification processed to assure the safety and security of its use.

All components employed have been tested by their companies and are certified as safe. However, the safety of the prototype itself would need to be evaluated performing a safety specific proof-of- concept and checking its compliance or determining the required fixes of errors.

Being the device designed categorized as a II a type medical device for being in contact with the patient, security measures apply, and keeping in mind the character of the device, electrical insulation is the main concern. In terms of the prototype, this has been taking into consideration, the casing is made in PLA, an insulating material,

and so are the rest of components that are in direct contact with the patient or technician that would conduct a test, such as the tubes and the mouthpiece.

Summing up this chapter, it can be concluded that the breath sampling device prototype is technically feasible, although some improvements may be performed.

8. Economic feasibility

The economic feasibility is assessed focusing on the tangible costs required to develop the devices. However, intangible costs and benefits are also taken into consideration

- **Tangible costs**

Tangible costs include the cost of material equipment, such as computers, as well as human resources expenses such as the cost of programmers 'time, and other employees 'salaries. These are the costs that will require a cash expense and are summarized in the following table:

Licenses needed to develop the software of the device are not considered as the university provides the required ones, or non commercial versions of the software can be used due to the fact that a prototype is developed, however, these could add about 1200 euros extra for the MATLAB and LabView licenses when a student fee is not applied.

Components	**Units**	**Price (EUR)**
Arduino UNO board	1	25
Laptop	1	800
SFM3000	1	123
M-EV-2-12	2	131.40
KNF NMP850KNDC	1	99,5
3014VD DC pump	1	84.8
PCB	2	100
Tedlar Bag	2	40
Mouthpiece	1	10
Non-rebreathing valve	1	30
25W Single Output Switching Power Supply	1	16,67
Case breath sampler	1	30
Case	1	15
Extra components	-	50
Shipping costs	-	33.48
Human Resources	**Hours**	**Price (EUR)**

Employee salary	300 h min	10 / h
Total		4563.85

Table 14. Estimated cost of the breath sampler prototype.

- **Intangible costs**

Given the difficulty to estimate intangible costs, no monetary amount is estimated for them when developing a medical device, nevertheless, they are considered factors in decision making along the process, for example, having a competitive edge in the market.

In contrast to tangible costs, there are many intangible costs. For example, the cost expenditure to improve patient satisfaction or discomfort when performing a respiratory test with the device, and training costs to instruct the technician to carry out the tests.
All these costs are in conjunction with many intangible benefits that are quite challenging to measure, such as patient satisfaction rate. Often, the benefits of these cost items cannot be directly measured, but these cost items are considered strategic costs or at a strategic level, and are taken into account in decision making along other factors. [81]

Intangible benefit ssuch as improving the patient's mood in front of the procedure, as it is non-invasive; can suppose a competitive edge in the market, and are considerend too when assesing feasibility.
Having said all this, it can be concluded that , the prototype itself could be considered low-cost and economically feasible, and that the biggest expense associated to it would be human resources, i.e. the salary of the employee performing the test.

9. Execution chronogram

The execution chronology is displayed from a visual approach by developing a PERT study and a Gantt chart, and in order to do so, all tasks and milestone are defined and related between them.

9.1. Timing and task definition

The final degree project, stablished to last 300h, is distributed in around 6 months. These hours are partitioned between hour of lab work, and hours planned to be done at home and with some tutorships from the supervisor.

The principal tasks are described by the three main objectives: develop a Breath Sampler, an accessory ambient air sampler, and performing a proof-of-concept. Nevertheless, to plan the schedule, subtasks are defined.

Task	Description
A	Research/project familiarization
B	Learning Arduino
C	Learning 3D CAD Modelling

D	Learning Design Spark
E	Hardware improvement-PCB
F	Environment sampler development
G	Hardware assembly
H	Software improvement
I	Planning Proof-of-concept
J	GC-MS familiarization
K	Performing Proof-of-concept
L	Analysis of results

Table 15. Task definition and description.

9.2. PERT

A Program Evaluation Review Technique (PERT) graph is the tool used to analyze and manage the tasks involved in the project completion timeline.

Duration is given by months takin into account that in a month there are 20 working days where 2-3 h of work is done.

As can be seen, some tasks depend on others while in the end the relations are linear, we must finish a task to start another one. If the critical path is studied, looking at the path were early and last time is equal, two of them, corresponding to the first two branches are detected, showing that most of the tasks are involved in the final time, so the project is quite strict in timing.

	Previous Activities	**Duration (unit of time)**
Research/projectf amiliarization	-	0,5
Learning Arduino	A	0,5
Learning 3D CAD Modelling	A	1
Learning Design Spark	A	0,5
Hardware improvement-PCB	D	1
Environment sampler development	B	1

Hardware assembly	C, E, F	2,5
Software improvement	A	2
Planning Proof-of-concept	A	0,5
GC-TCD familiarization	I	0,5
Performing Proof-of-concept	H, I, J	0,5
Analysis of results	K	0,5
Writing Project	L	0,5

Table 16. Sequence and time of the project tasks.

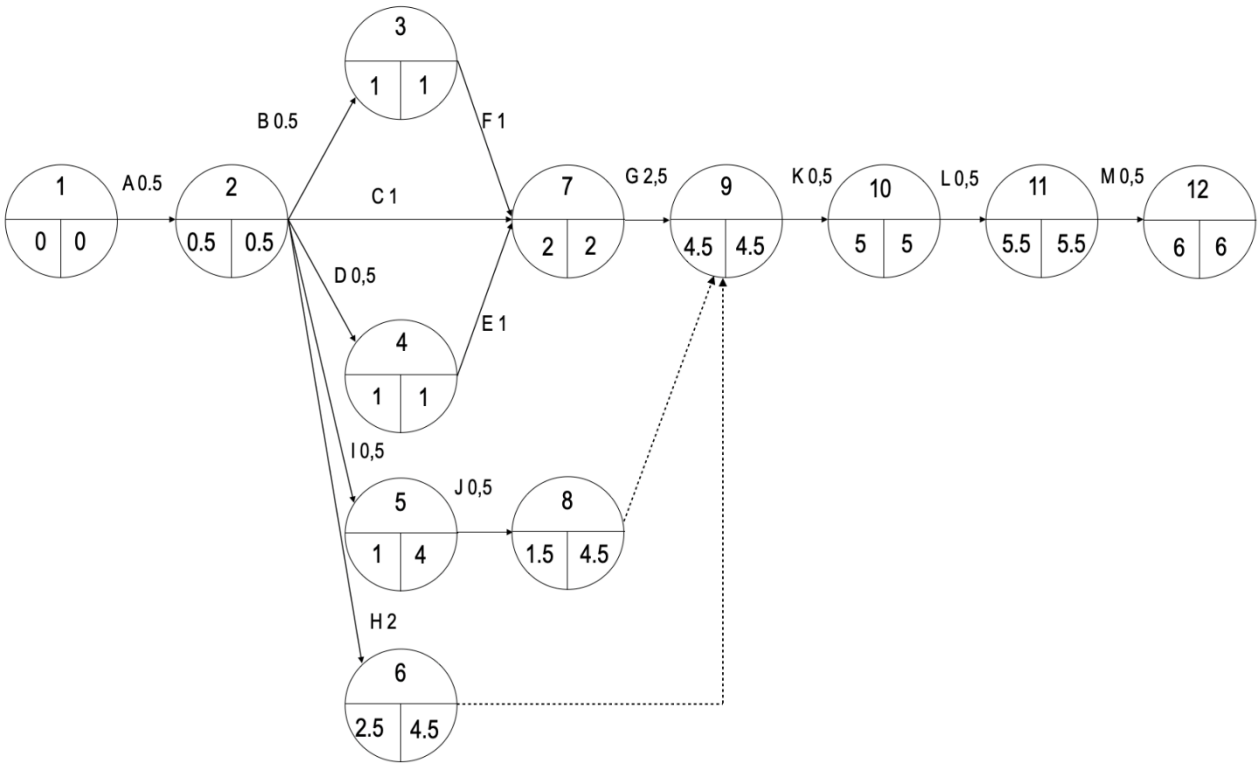

Figure 26. PERT Diagram.

9.3. Gantt chart

Finally, a Gannt of the real timing is displayed to track project schedules and visualize how the tasks related to each other. It is observed that not all tasks carried out were planned, as they proceed from the redesign that was needed, and this also defined the timing of the execution.

Figure 27. GANT diagram of the project.

10. Budget

This section reflects all costs associated to the development of the present project.

In the breakdown of the budget of a project two main categories star in: human resources and equipment.

Concerning the first one, because of the development being made by a student and no income is receive, there are no costs associated to it.

Secondly, the equipment category includes both hardware and software. All these hardware costs are detailed in Table 17.

	Unit Cost (EUR)	Units	Total (EUR)	Execution cost (EUR)
HARDWARE				
Laptop	800	1	800	0
Arduino UNO board	25	1	25	0
Eleego Uno R3	10	1	15	0
SFM3000	123	1	123	0
M-EV-2-12	65.70	2	131.40	131.40
KNF NMP850KNDC	99.5	1	99.5	0
3014VD DC pump	84.80	1	84.8	0
PCB	50	2	100	0
Tedlar Bag	20	2	40	0
Mouthpiece	10	1	210	10
Non-rebreathing valve	30	1	30	0
25W Single Output Switching Power Supply	16.67	1	16,67	16.67
Case breath sampler	30	1	30	0
Case	15	1	15	0
Extra components[10]	50	-	50	25
OTHER COSTS				
Shipment costs M-EV-2-12	33.48	-	33.48	33.48
Total	1468.05		1603.75	216.55

Table 17. Real execution cost of the project.

Some components were needed such as terminal blocks, pin strips, new rubber for the sealing of the vacuum chamber, as well as tubing.

It must be mentioned that although costs in the previous table express the cost per unit, the items listed will not be used just once, so we could calculate instead of this a 20% as the amortization percentage of the overall price to be the actual cost of the prototype, being 360.75€ instead of the 1803.75€ estimated. Also, it is specified in the table the cost of the overall components of the prototype, and the execution cost of this project in the last column, being this the real expense allocated in the development of the breath sampler in the context of the present project. Also, as the majority of these component costs mentioned were already owned, such as laptop, PCB etc, it would not reflect the real cost.

Concerning the software all licenses used in the project are financed by the UB, free or have free educational versions and all 3D printed structures were finance by UB and printed in the Electronics Department's workshop.

The estimated cost of the project was around 250 € when it started, and less has been needed to develop it. This means that, the project can be developed normally inside the budget limitations.

11. Conclusions

Breath analysis is a hopeful tool for the diagnosis and monitoring of patients affected by a wide range of diseases, but some hurdles hamper the development and application into the medical field of this low invasive technology, being one of them the lack of standardized procedures to acquire the breath samples.

A breath sampler prototype has been built with the main goal in mind of protocolizing the obtention of exhaled breath samples, and a proof of concept has been performed to test its functioning.

Consecutively, the objectives set for the project in section *1.2.Objectives* are reviewed to check its fulfillment.

- Review of current literature on breath analysis and breath sampler devices

The familiarization with the principles of breath analysis and the devices developed for the purpose of sample collection was fundamental for the understanding of the project and for setting the objectives expected by its development. It was performed as the first task of the project as well as along the process, when research was needed to solve the arising issues and in the writing of the report. This point is accomplished and reflected, as it can also be seen in the section *2.2 State of the art.*

- Development of a hardware system and designing a compact case structure to put together a cost competitive breath sampler device

The hardware of the system had to be reviewed and redesigned with reference to the previous prototype. It was then assembled using a PCB to connect all electrical components. Previous to the final assembly, the prototype was built and a case was created to contain it, but due to the corrective redesigning that it was required in order to make it functional, both the PCB and the case became obsolete, and new ones had to be implemented, which was successfully achieved as seen in *6. Detail engineering stage.*

Moreover, when analyzing the final prototype that has been obtained, it can be said that it is a competitive-cost breath sampler device, as seen in Budget. It must be noted that future improvements can be carried out concerning the hardware, for example, the closing system of the vacuum chamber could be replaced by a snap-

release rubber closure that would reduce the time consuming task of screwing and unscrewing the bolts each time the chamber has to be opened or closed.

- Implementation of software to be used through an user friendly interface in order to achieve the desired functionalities for the hardware, with evidences found in section *6.3.1 Breath Sampler software.*

- Development of a complementary device to obtain environment air samples to be used as a reference for breath sampler's samples analysis

Hardware and software were successfully implemented to achieve a sample collector of ambient air, as seen in *6.2.2.and 6.3.2 Ambient air sampler.*

- Implementation of software to be used through an user friendly interface in order to achieve the desired functionalities for the hardware

Firstly, the code developed in the first prototype was reviewed and studied. When trying to operate with the Labview user interface, it was seen that it was not functional, and some adjustments were made. It also had to be adapted to work with the redesigned hardware system. Then, new functionalities were implemented, and an updated and extended interface developed, as reflected in *6.3. Software.*

- Performing a proof of concept of the breath sampler

The performance of a proof of concept was carried out in the Department of Electronics, as it was detailed in section *6.5 Proof of concept.*

Some trials with the GC-TCD device were performed using exhaled breath as well as a mixture of methane, CO2, and nitrogen which were detected and validated the expected and proper functioning of the device.

A more accurate analysis of the sample collected in the Tedlar bag could be done, but this was limited due to the analytical equipment in the lab. Also, it must be taken into account that the results proceed from a single subject and a simulation, and future studies with a group of real patients to explore in depth the variability of their breath samples would be of great interest. Future improvements of hardware as implement a CO_2 sensor or code would be also welcome.

It is worth mentioning the fact that this project has been a great learning experience, as it has covered diverse fields as electronics, CAD modelling, and embedded software while binding all with its medical application. It has been also of interest and usefulness to learn about project development, autonomous work and task organization.

Summing up, a breath sampler has been developed and assembled in order to acquire in a protocolized manner breath samples, useful to diagnose and monitor patients non-invasively. A proof of concept has been carried out successfully, validating its functioning and therefore obtaining a low-cost breath sampling device prototype.

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Annexes

Additional information related to the Breath Sampler prototype development project are found in this document.

1. Arduino IDE Code

The code developed for the functioning of the ambient air sample is displayed hereunder commented.

```
/*The goal of the following code is to get an ambient air sample and store it in a Tedlar bag.
Selection and actioning a switch button in function of the bag sizing, the device is executed
for a defined time, and stopped afterwards
*/

const int led1 = 8; //led pins
const int led2 = 9;

const int pump = A5;//pump pin

const int button1 = 10; //button pins
const int button2 = 11;



unsigned long starttime1; //declaration of variables to use in timer function inside if condition
unsigned long endtime1;

unsigned long starttime2;
unsigned long endtime2;



void setup() {

  //setup code, to run once:
  pinMode(button1, INPUT); //pins set up
  pinMode(button2, INPUT);

  pinMode(led1,OUTPUT);
  pinMode(led2,OUTPUT);

  pinMode(pump, OUTPUT);


}

void loop() {

  //main code, to run repeatedly:
  if(digitalRead(button1) == LOW){

    starttime1 = millis(); //internal timing from board stored in variable
    endtime1 = starttime1;

    while ((endtime1 - starttime1) <= 30000){  //execution time in miliseconds compared to the time from the moment it
      digitalWrite(pump, HIGH); //pump and led executed while the condition is true
      digitalWrite(led1, HIGH);

      endtime1 = millis(); //stores time reference from board one period of time defined has passed

      }

    digitalWrite(pump, LOW); //activation of components stopped whne period of time is over
    digitalWrite(led1, LOW);
    }


  if(digitalRead(button2) == LOW){ //same structure as above, for the other button with different timing period
```

```
60
61    starttime2 = millis();
62    endtime2 = starttime2;
63
64    while ((endtime2 - starttime2) <= 60000){ // 60 seconds time period
65      digitalWrite(pump, HIGH);
66      digitalWrite(led2, HIGH);
67
68      endtime2 = millis();
69
70      }
71
72    digitalWrite(pump, LOW);
73    digitalWrite(led2, LOW);
74
75    }
76
77 }
```

2. Proof of concept R plotting

The plots shown in the Proof of concept section were obtained with the following code. It must be mentioned that although signal processing was out of the scope of the present project, it would be of great interest and utility for studies of samples acquired with the prototype in detail and its further development.

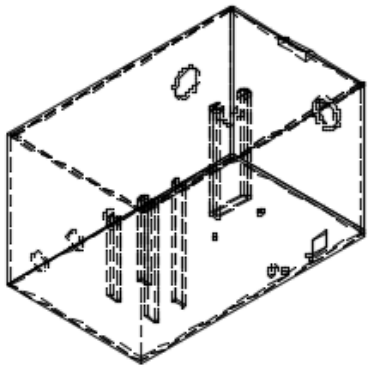
```
1  library(dplyr) # package that provides tools for efficiently manipulating datasets
2  library(plotly) # graphing library
3
4  datos <- read.table("C:/Users/Biosignal/Downloads/P08.txt", header = FALSE, sep = "\t", dec = ".", skip = 43) %>%
5    select(1,3) %>% # Middle column is a constant value due to method selection in the GC-TCD, not of interest
6    rename('Time' = 1, 'Value' = 2) #Data columns labelled
7
8  # %% operator used allows to chain operations from left to right
9
10 plot <- plot_ly(
11   datos,
12   x= ~Time,  #Columns asigned to axis
13   y = ~Value,
14   type = "scatter", #Type of plot and mode of plotting assignation
15   mode = 'lines') %>%
16   layout(title = "Injection 05", # Graph title
17          showlegend = FALSE,
18          xaxis = list(title = 'Time (min)'), # Axis labels
19          yaxis = list(title ='Value (mV)'))
20
21 plot
```

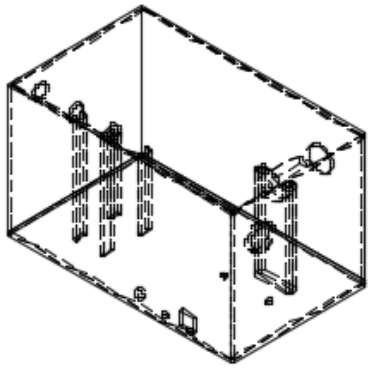
In addition to the detection data acquired reflected in the output file of the chromatogram, there is also other data stored in the file. This information is shown in the next figure.

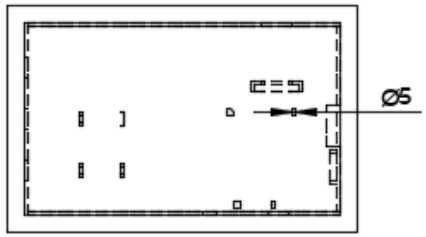
```
File Path       chrom://isp_lab-pc/ChromeleonLocal/Instrument Data/TRACE_1300/GASES/
breathsampler/Prueba con Ar.seq/407.smp/FrontDetector.channel/FrontDetector.chm
Channel FrontDetector

Injection Information:
Data Vault      ChromeleonLocal
Injection       test_bolsa_Ar
Injection Number        8
Position        7
Comment
Processing Method
Instrument Method       prova aire sintetic rampa rapida3
Type    Unknown
Status  Finished
Injection Date  17/05/2021
Injection Time  16:44:28
Injection Volume (µl)   1.0
Dilution Factor 1.0000
Weight  1.0000

Chromatogram Data Information:
Time Min. (min) 0.000000
Time Max. (min) 14.400000
Data Points     8641
Detector        GC
Generating Data System  Chromeleon 7.2.4.8179
Exporting Data System   Chromeleon 7.2.4.0
Operator        Instrument Controller
Signal Quantity
Signal Unit     mV
Signal Min.     -1.327114
Signal Max.     87.721893
Channel FrontDetector
Driver Name     Trace1300Driver.dll
Channel Type    Evaluation
Min. Step (s)   0.1
Max. Step (s)   0.1
Average Step (s)        0.1

Signal Parameter Information:
Signal Info
```

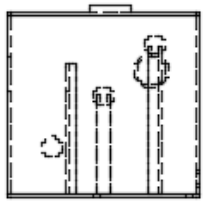
3. Case

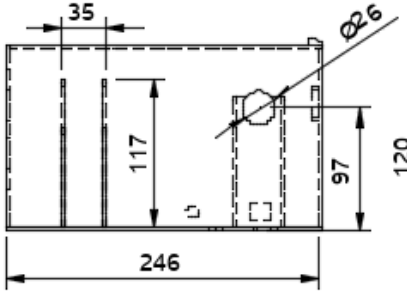
The dimensioned 3D model views drawings of the cases designed to assemble all components of the samplers together are displayed in the next two pages of the document.

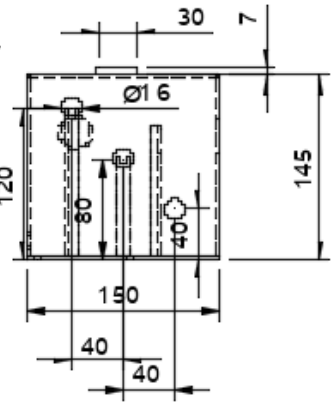


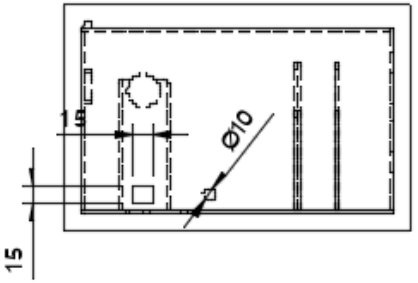


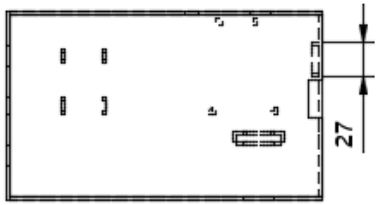


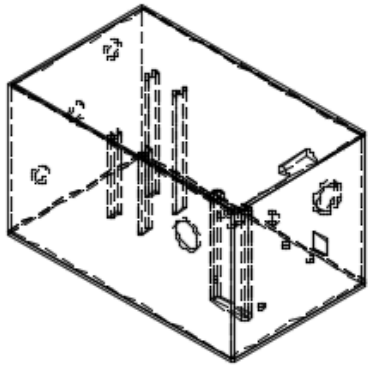




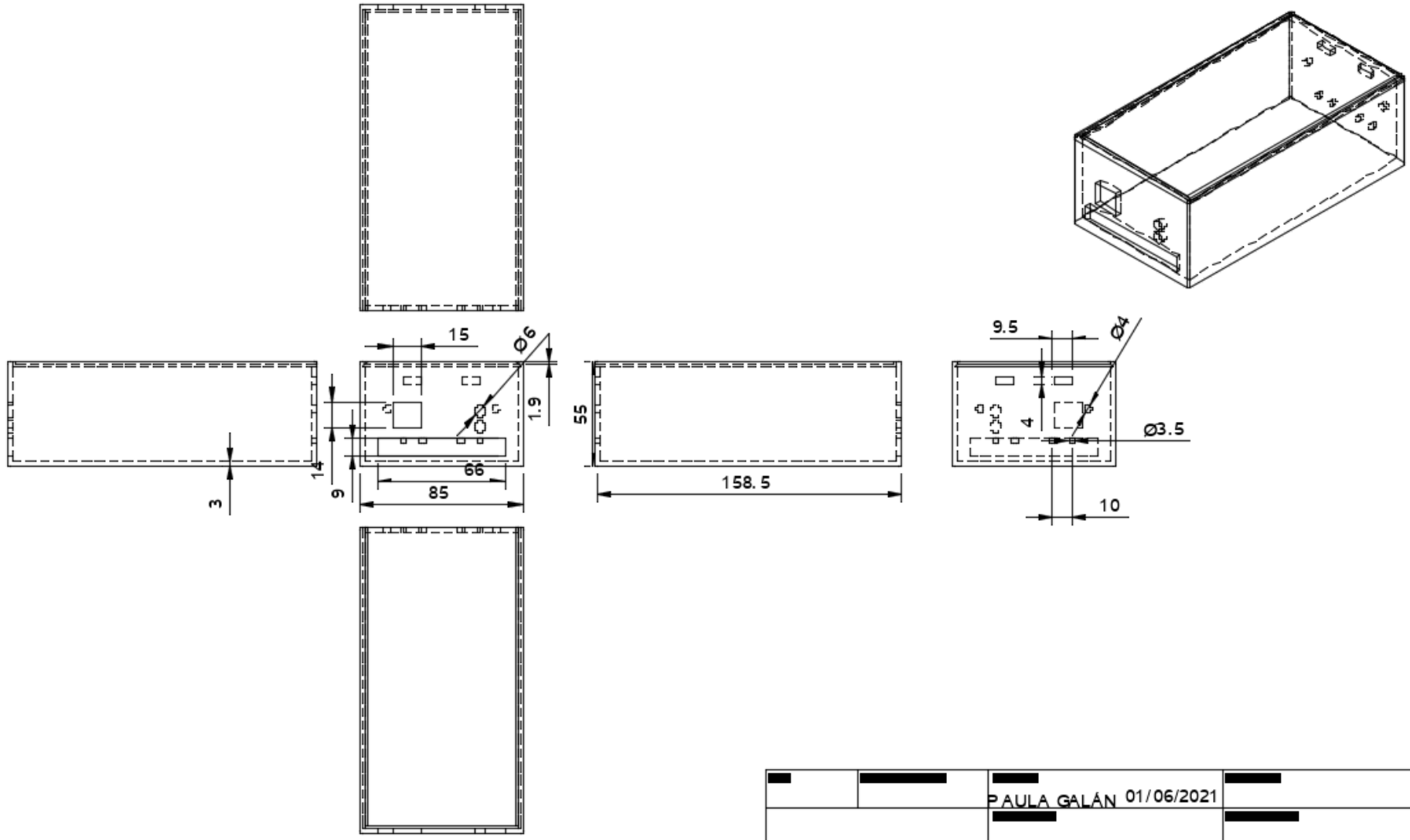








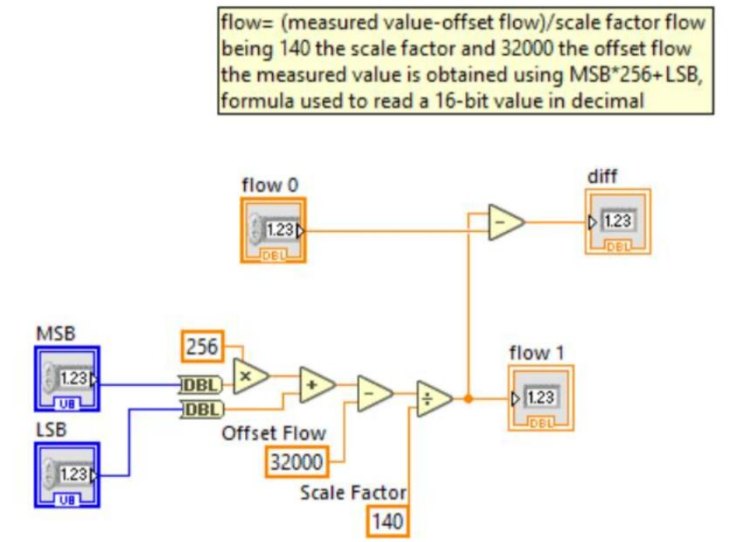
		PAULA GALÁN 01/06/2021	
		Breath Sampler Case	
			1/1

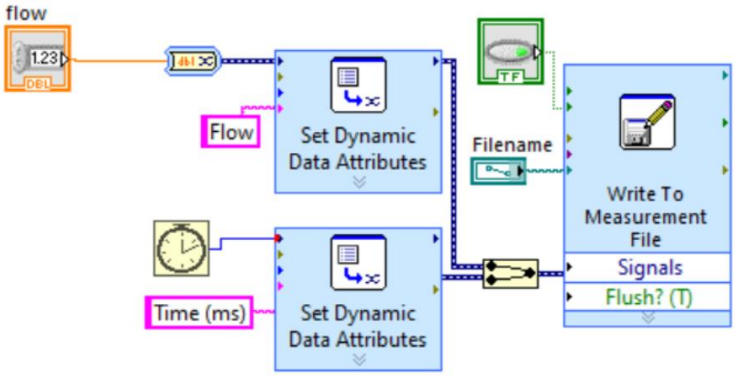


		PAULA GALÁN 01/06/2021	
		Ambient Air Sampler Case	
			1/1

4. LabVIEW

Two subVI, one to calculate the flow and a second one for saving the data into a file, that are a subdivided part of the main block diagram of the Breath Sampler LabVIEW program are found here.





Zoomed versions of the figures in section 6 are provided too.

Select a working mode:

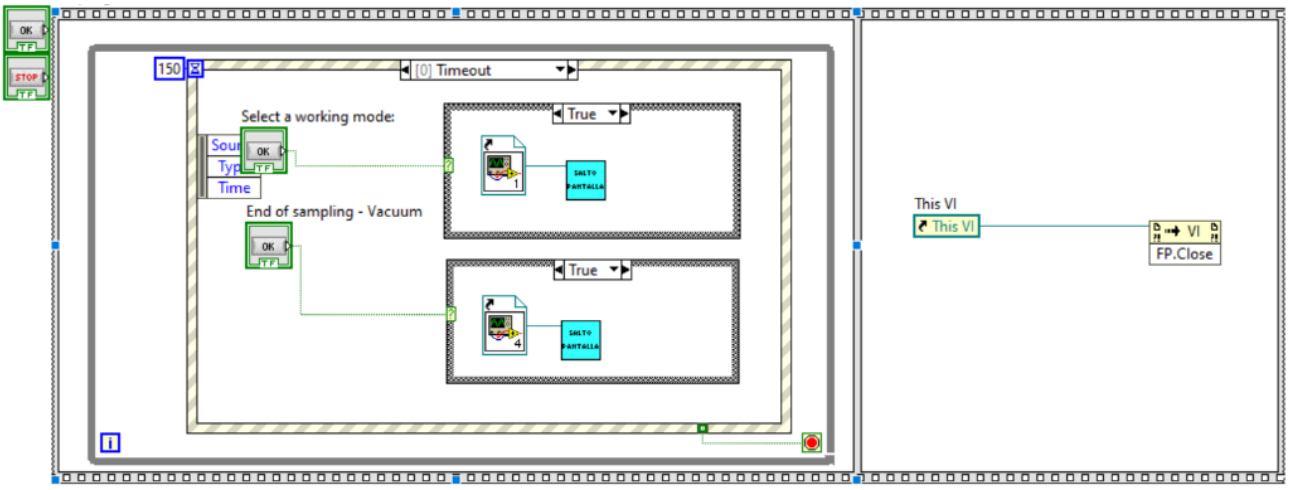
Breath Sampling

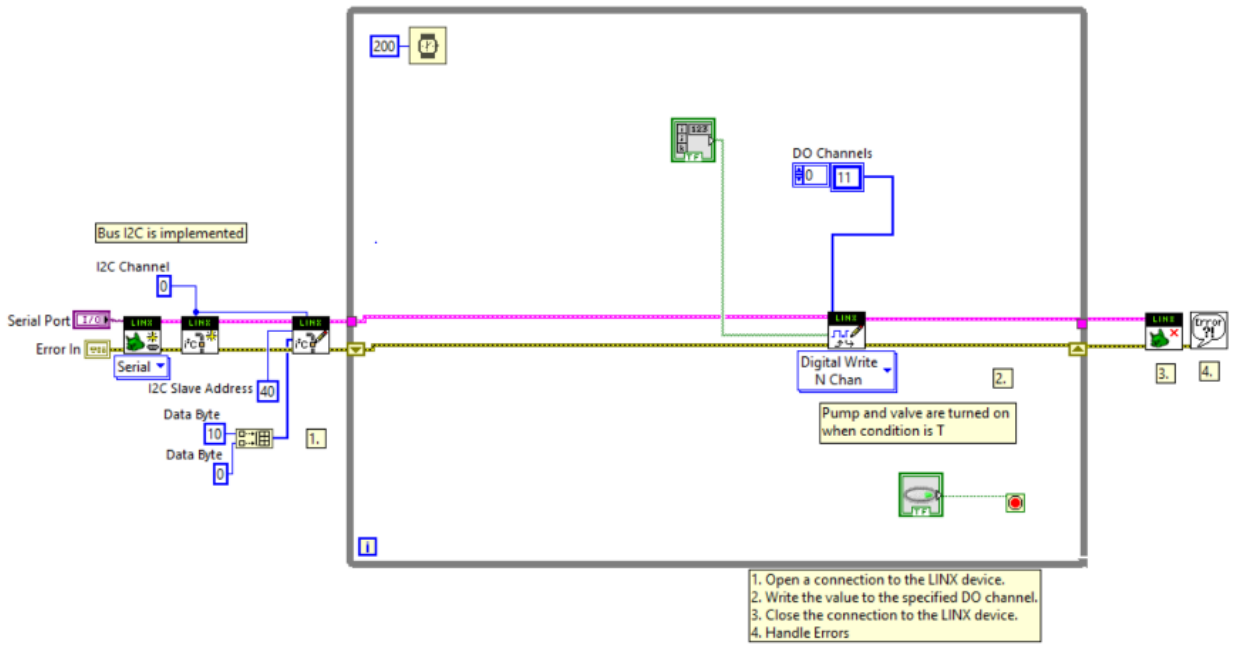
Vacuum release

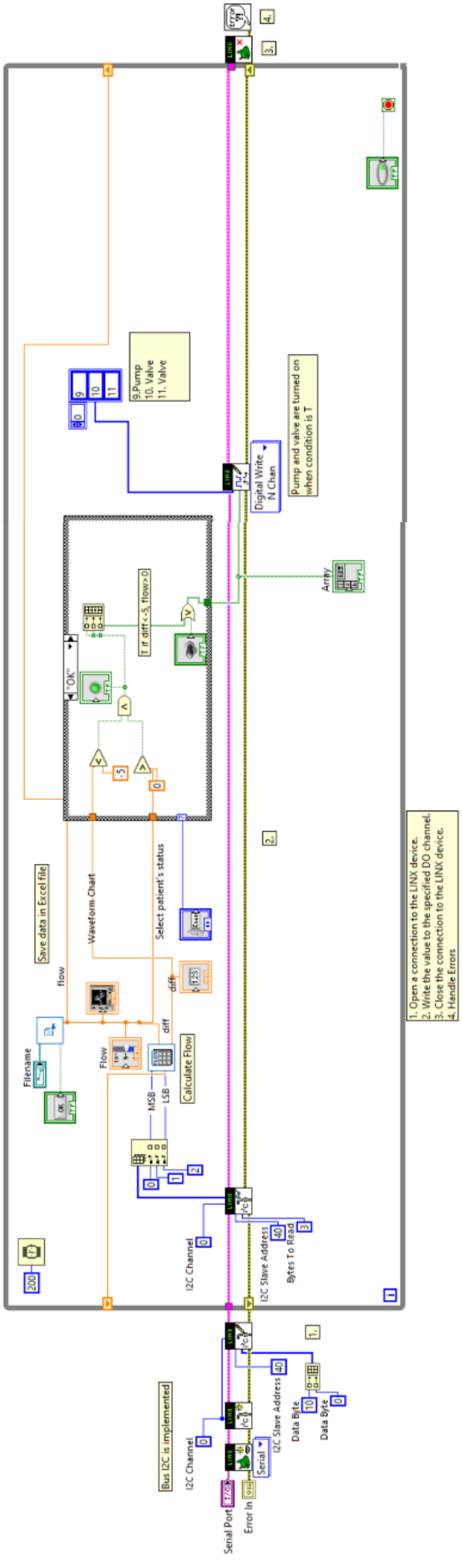
EXIT

Press button to release the vacuum

RELEASE VACUUM

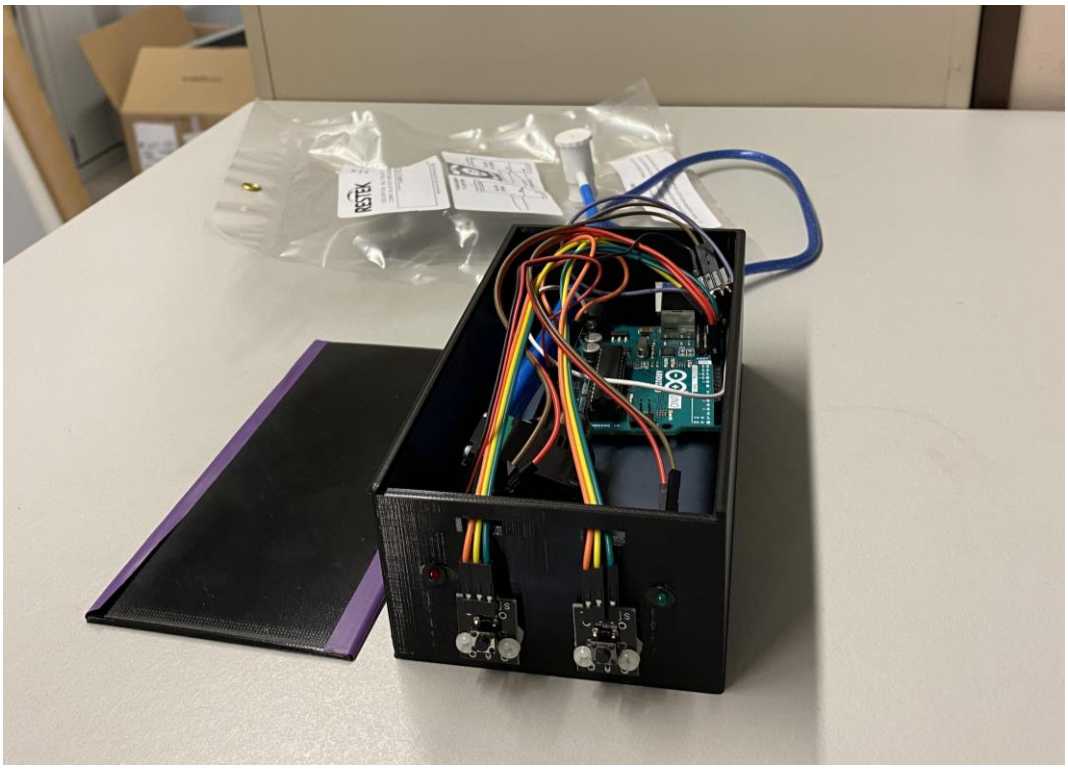


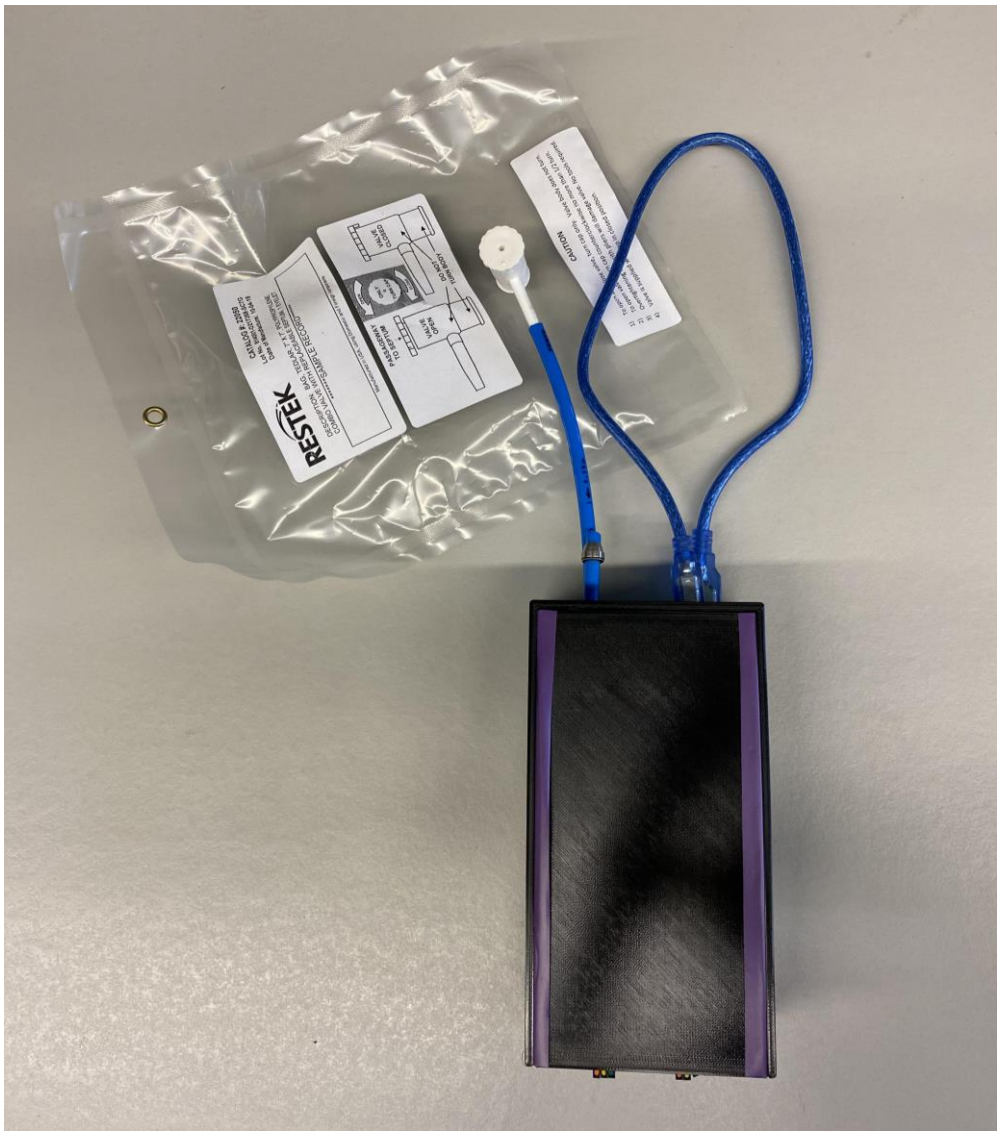



9.Pump
10. Valve
11. Valve
Digital Write
N Chan
Pump and valve are turned on
when condition is T
Array
T if diff <-5, flow> 0
"OK"
-5
0
Save data in Excel file
Waveform Chart
Select patient's status
flow
diff
Filename
Flow
MSB
LSB
Calculate Flow
I2C Channel
I2C Slave Address
Bytes To Read
200
1. Open a connection to the LINX device.
2. Write the value to the specified DO channel.
3. Close the connection to the LINX device.
4. Handle Errors
Bus I2C is implemented
I2C Channel
I2C Slave Address
Data Byte
Data Byte
Serial
Serial Port
Error In

5. Ambient Air sampler

Evidences of the final assembly of the ambient air sampler are displayed hereafter.




RESTEK
CAUTION