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Implementation of Fowler's method for end-tidal air sampling

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Abstract

The design, realization and testing of a CO_2-triggered breath sampler, capable of a separate collection of dead space and end-tidal air on multiple breaths, is presented. This sampling procedure has advantages in terms of the sample volume, insights regarding the origin of compounds, increased reproducibility and higher concentrations of compounds. The high quality of design and the speed of the components ensure a breath-by-breath estimate of dead volume, as well as the comfort and safety of the subject under test. The system represents a valid tool to contribute to the development of a standardized sampling protocol needed to compare results obtained by the various groups in this field.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Despite its enormous potential, breath analysis is far from being widely used in clinical practice. A well-argued list of reasons for this has been put forward by Risby and Solga [1]. However, from a technical point of view, the lack of established sampling and measurement procedures for the analysis of volatile compounds at a trace level plays a key role.

Exhaled air can be sampled in two ways: mixed expiratory sampling and end-tidal sampling. Mixed expiratory sampling entails collecting total breath, including the air contained in the upper airways which experiences no gas exchange with blood (dead space). End-tidal sampling involves the collection of only end-tidal air, which contains most of the chemical information on blood composition.

Separate sampling during expiration of dead volume air and end-tidal air may be advantageous in breath research in terms of providing an insight into the origin of chemicals identified in breath samples. The presence of exogenous compounds in breath is one of the main sources of noise affecting breath analysis research results, and how to discriminate between compounds with endogenous (i.e. produced inside the body by the physiological or pathological metabolism) or exogenous origin is an age-old question. The discussion on the best solution to this problem still divides the scientific community. There are those that propose that each compound should be weighed on the basis of its concentration gradient between breath and ambient air [2], those that maintain that compounds whose concentration in ambient air is comparable to or higher than in breath should not be taken into account in the characterization of subjects [3] and, finally, those that provide the subjects with purified air in order to circumvent the problem [4, 5]. In fact, this last solution is also disputable. Exogenous compounds reaching the lungs dissolve in blood according to their blood/air partition coefficient and are then transported throughout the body, dissolving into different tissues on the basis of chemical affinity. The concentration of every single compound in alveolar air is hence the result of a dynamic equilibrium

involving many compartments, each with its specific time constant. Interactions between different compounds cannot in principle be ruled out and are known to happen, such as the case of the competition of oxygen and carbon monoxide for binding hemoglobin. As a result, it has been proved that different compounds have different washout periods ranging from minutes to hours [6], which restricts the usefulness of purified air delivery for the removal of exogenous compounds to a very limited number of cases. Furthermore, exogenous water-soluble compounds can also dissolve in the mucus layer covering the mucosa of the conducting airways, thus making the overall picture even more complex. This effect has been reported by developers of pharmacokinetic models, who were unable to provide correct mass balances without taking it into account [7]. At the same time, some endogenous breath components of clinical interest such as nitric oxide are known to be produced in the conducting airways [8], and others may be found in future. All these considerations lead to the conclusion that separate sampling of different breath fractions could provide valuable help to breath research. When interest is focused on blood/air gas exchanges at an alveolar level, a further advantage of the separate sampling is that it avoids the dilution of end-tidal with dead space air. Such dilution varies among different individuals and also in the same individual depending on sampling conditions, so its variability can prevent the correct quantification of compound concentrations in end-tidal air and alter the multivariate data patterns obtained in the measurement campaigns. Better reproducibility of data is obtained when only the end-tidal fraction of breath is analyzed [9]. Compounds with an alveolar origin give higher concentrations in end-tidal than in mixed expiratory samples. This is a positive effect since low concentration levels of breath markers represent a constant analytical challenge [10].

Several types of breath sampling devices have been reported in the literature or are commercially available. Most are simple combinations of valves and connections which the study subject uses to fill a sampling bag or a syringe [11–13]. Pleil *et al* sample breath in pre-evacuated stainless steel canisters [14]. The patients themselves control the sampling by opening a manual valve through which their breath is sucked into the canister. In all these cases, the study subject breathes ambient air and mixed expiratory sampling is accomplished. Other authors have suggested more complex devices to deliver purified air to their subjects [15, 16]. Two passive devices are commercially available that allow the sampling of end-tidal air. Quintron's device consists of a tee-shaped connector housing two one-way valves in its two outlets. The core of each valve is a thin silicon disc that seals the outlet until threshold pressure is exerted. The two valves are regulated to open at different pressures; a 250 ml bag is connected to the valve opening at the lower pressure, while a 750 ml bag is connected to the other valve. A mouthpiece is fitted to the inlet of the tee-shaped connector. When the subject blows air into the device, the pressure rises in the connector until the first valve opens and the corresponding bag is filled with dead space air. The pressure then starts increasing again until the second valve opens, allowing the remaining breath to be released into the other bag. Another sampler, BioVOC^{TM}, is produced by Markes International. The subject is asked to blow through a mouthpiece into a cylinder with an open end. Only the last portion of end-tidal air (150 ml) remains in the cylinder after expiration. The mouthpiece is then replaced with a piston used to push the sample through an absorbing tube which has been connected to the previously open end of the cylinder. Although these systems do not provide the optimum solution, they are nevertheless attractive in terms of their simplicity and low cost. Their main limitations are the poor control of the sampling conditions and the limited volume that can be sampled in a single breath. A more sophisticated breath collecting apparatus was developed by Phillips and produced by Menssana Research [2]. In this system, the subject breathes through a mouthpiece assembly consisting of an inlet valve for the inspiration of ambient air and an outlet valve connected to an open-ended stainless steel cylindrical reservoir, which is thermostated at 40 °C to avoid the condensation of water. The sampling port, located at the end of the reservoir near the mouthpiece, is connected in sequence to an absorption tube, a flow meter and a pump. A microprocessor controls the system, switching the pump on when necessary. The selection of end-tidal air inside the reservoir is obtained by activating the pump at appropriate times after expiration, since no measurements of expiratory gas concentration and flow are taken. The total volume sampled in the absorbing tube during multiple breaths can be selected by the user. A CO_2-controlled breath sampling device was proposed by Schubert *et al* for mechanically ventilated patients [17]. A fast responding infrared absorption mainstream CO_2 analyzer supplies data to an electronic processing unit, which actuates a two-way valve diverting breath flow to an absorbing trap when the percentage volume of carbon dioxide exceeds the set-up point.

The system presented in this study is based on the same philosophy, but breath is sampled into a bag and the pre-concentration of analytes in an absorption tube is then performed off-line. The system integrates a flow meter and a carbon dioxide measurement unit based on wavelength modulation spectroscopy. Flow direction is controlled by a system of valves that can be triggered by the attainment of either volume or carbon dioxide concentration values. A breath-by-breath calculation of the volume of anatomical dead space is achieved by an algorithm implementing Fowler's method, while end-tidal CO_2 concentration values can be inferred from the CO_2 concentration data. The system enables large sample volumes to be sampled on multiple breaths.

2. Physiology of respiration and discrimination of breath fractions

A typical respiratory cycle in a time span of about 5 s involves the exchange of half a liter of air with the lungs (tidal volume), so that the total ventilation, i.e. the volume of air moved in and out of the lungs per unit of time, is about 6 l min^{-1}. Not all the air we breathe is useful for the renewal of respiratory gases. The respiratory system can be divided into a section, namely dead space, which mainly acts as a conducting airway (nose, pharynx, larynx, trachea and other airways

without alveoli), and a section whose chief function is gas exchange (alveoli and alveolar sacs). The typical ventilation per respiratory cycle of these volumes is 150 ml and 350 ml respectively. Before inspiration, dead space is filled with end-tidal air remaining from the previous respiratory cycle. Then, during inspiration, half a liter of fresh ambient air is sucked into the body, but only the first 350 ml reach the lung together with 150 ml of end-tidal air contained in the dead space, where together they are diluted and mixed with alveolar air. During expiration, 150 ml of ambient air, which filled the dead space, and 350 ml of air coming from the lung are emitted through the nose and/or mouth in sequence. By analyzing a respiration cycle, it can be noted that dead space is alternately filled with ambient and end-tidal air, and that only 350 ml of ambient air actually ventilates the lungs. Since the volume of air contained in the lungs during normal breathing is approximately 3 l, it follows that the composition of alveolar air is pretty stable during respiration (the cyclic variations of oxygen and carbon dioxide are about 2% and 5%, respectively).

Each breath sample is thus made up of many different 'airs', each adding its particular content of chemical information. Ambient air is at the same time the main source of xenobiotic contaminants and oxygen as well as being a sink for carbon dioxide and endogenous compounds. Its content of oxygen and carbon dioxide is fairly constant, while the concentrations of xenobiotics may be highly variable. Dead space air has a composition close to ambient air (but a higher water content). Differences may arise due to chemicals originated and/or released in the conducting airways or to gas exchanges with the mucus layer (mainly for water soluble compounds). Alveolar air is the air contained in the lungs. Its composition is due to the interaction of ambient air with blood through the alveolar membrane. There are considerable non-homogeneities in the composition of alveolar air among different lung regions even in healthy subjects, since posture and gravity alter both local ventilation and perfusion, i.e. the blood flow through the alveolar vessels. End-tidal air is the last fraction of expired air, whose composition resembles alveolar air (slight differences arise from partial mixing with dead space air). The breath sample collected using a mixed expiratory sampling technique is then a mixture of dead space and end-tidal air. When the composition of ambient air changes, the time needed for each breath fraction to reach a new equilibrium increases deeper inside the body, ranging from a few seconds for dead space air to minutes or hours for alveolar air.

Which breath fraction should be considered as the most representative of an individual's conditions?

There is no general answer to this question, it depends on where the chemical markers are released into the breath. In most cases, blood is the main source of marker and for this reason the average alveolar air should be considered the best option. In this case, a sample obtained by collecting the end-tidal air from many multiple quiet breaths would be the closest approximation to the ideal solution, since this procedure averages breath-by-breath fluctuations in composition due to the irreproducible lung emptying (air is expelled at different moments from the various lung regions) and flow variations. If markers are released from conducting airways, dead space air should be sampled.

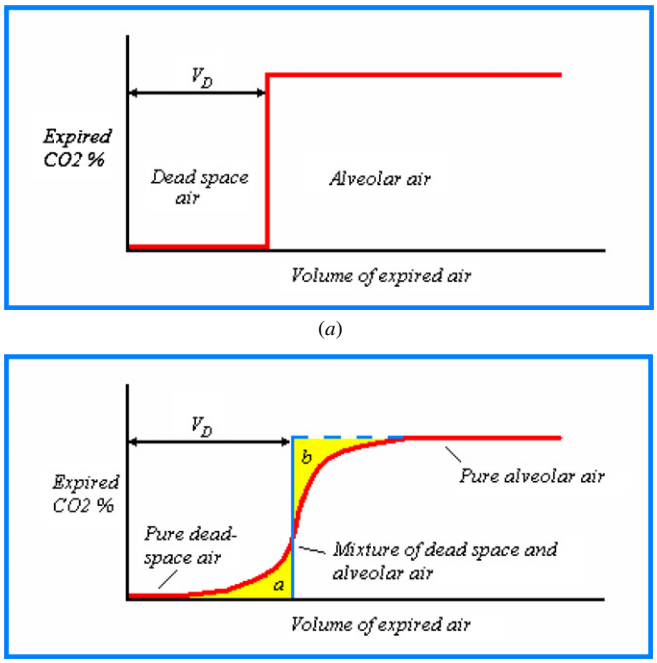

Figure 1. (*a*) CO_2 concentration versus expired volume in the absence of mixing between dead space and alveolar air (ideal case) and (*b*) actual CO_2 concentration profile in the case of mixing. Fowler's graphical method is shown: the dead space volume corresponds to the point where the shaded regions *a* and *b* have the same area.

Dead space and end-tidal air can be distinguished on the basis of their composition. Unfortunately, a net separation cannot be made between the two due to the absence of a sharp anatomical boundary. A boundary region has been identified at the terminal bronchioles with a 2 mm diameter, where the convective flow gives way to diffusion [18]. The diffusion and mixing of gases in this region depend on their physical–chemical properties, on the rate of respiration and on the anatomy. The concentration of expiratory gases is also greatly influenced by the ratio of ventilation-to-volume, perfusion-to-volume and ventilation-to-perfusion inequalities [19], as well as lung volume [20], posture [21] and flow rate [22]. Hence, it is not surprising that breath samples collected under different conditions show differences in composition [23]. As carbon dioxide is virtually absent in atmospheric air while having an average partial pressure of about 40 mmHg in an ideal lung, a step function profile would be observed for this gas in expired air, if no mixing had occurred between dead space and end-tidal air (figure 1(*a*)). Thanks to the presence of different path lengths of dead space between the mouth and alveoli, the lack of a uniform velocity across the cross section of the tracheobronchial tree, and due to convective and diffusive mixing of gases at the boundary region, the transition in expired air is not so sharp, so that the concentration profile is rather S-shaped (figure 1(*b*)). In 1948 Fowler defined dead space as the volume of conducting airway as far as the location where a large change in gas composition occurs, and he proposed a method for determining its value [24]. This method consisted of giving the subject a single breath of pure

oxygen and monitoring the nitrogen concentration in exhaled air. The nitrogen concentration profile in exhaled air resembles the carbon dioxide profile reported in figure 1(*b*). The volume for which the shaded areas *a* and *b* are equivalent represents the ideal transition point between dead space and alveolar air. Fowler used nitrogen as a tracer gas because he had a fast sensor to measure its concentration levels in the expired air. Other gases, such as oxygen, carbon dioxide or helium, provide similar results if diffusivities are comparable [25, 26]. A different method based on a mass balance had been proposed at the end of the 19th century by Bohr using carbon dioxide as a tracer gas [27]. His formula was the expression of a simplified model that considered the expired breath as being the sum of two unmixed homogeneous fractions, the inspired air contained in the dead space and the alveolar air coming from the lungs:

$$V_E \times C_E = (V_E - V_{DS})C_A + V_{DS} \times C_{DS}, \quad (1)$$

where V_E and V_{DS} are the volumes of expired and dead space air, C_E is the average concentration of carbon dioxide in expired air (the value that would be measured in a sampling bag), and C_{DS} and C_A are the concentrations of the same gas in dead space and alveolar air respectively. The contribution $V_{DS} \cdot C_{DS}$ of dead space air to the carbon dioxide balance can be neglected as the concentration of this gas in ambient air is close to zero. If the carbon dioxide concentration C_A in the alveolar air is known (it is reasonably close to the end-tidal CO_2 concentration level), Bohr's formula can be rearranged and used to estimate either the dilution factor D of the alveolar air due to dead space air:

$$D = \frac{V_E}{V_A} = \frac{V_E}{V_E - V_{DS}} = \frac{C_A}{C_E} \quad (2)$$

or the dead space volume:

$$V_{DS} = V_E \left(1 - \frac{C_E}{C_A}\right). \quad (3)$$

Even though they provide about the same estimate of dead space volume in healthy individuals, there is an important conceptual difference between Bohr's and Fowler's approaches, originating from the use of tracer gases such as carbon dioxide and nitrogen which are involved or not involved in the respiration process, respectively. Fowler's method estimates the volume of the conducting airways to the point where the pure oxygen delivered to the subject mixes with the nitrogen in alveolar air (anatomic dead space), while Bohr's method measures the volume of air not involved in the gas exchange with blood (physiological dead space). The difference is due to the alveolar dead space, i.e. the volume of the lung constituted by alveoli that are ventilated but insufficiently perfused (or perfused but poorly ventilated) for gas exchange to be effective. Such volumes are normally very small (less than 5 ml) in healthy individuals, but can increase dramatically in patients affected by lung diseases characterized by ventilation or perfusion impairments such as bronchitis, emphysema or pulmonary embolism. Thus, if Bohr's method is important to clinicians in order to assess the alveolar dead space, at the same time the estimate of the dilution factor may be used by researchers in breath analysis to normalize data and increase reproducibility.

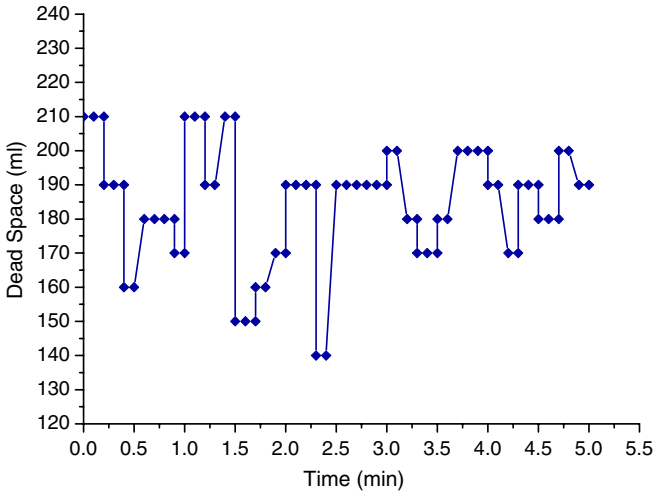

Figure 2. Dead space volume variations as recorded in a 5 min measurement session by a spirometer. A session characterized by extreme variability was chosen to show to what extent dead space can change during uncontrolled breathing.

3. Sampler design and realization

3.1. Specifications

It is challenging to develop a sampling device capable of discriminating between the dead space and end-tidal fractions due to a series of demanding constraints. Minimum head losses (a few ml of water) are required so that subjects do not have to make any effort when blowing, and the subjects' safety has to be ensured from a bacteriological and electrical point of view. Suitable materials have to be chosen to limit the contamination of breath samples, both in terms of the release of volatiles from the wetted parts of the various components of the device and in terms of memory effects related to the cross contamination between different samples (absorption/desorption of breath components onto wetted surfaces). The whole system has to be thermostated at a temperature higher than 37 °C to avoid condensation of large amounts of water vapor contained in breath. Fast sensing and processing units have to be used to control the valves that divert the breath into different sampling bags at the right moment. Exhalation during quiet breathing lasts an average of about 2 s, which is very little time to acquire data, make calculations and activate the valves. Ventilation patterns may easily change if experimental conditions are not set up to help the study subjects maintain a regimented breathing pattern. It has been shown that tidal volume and minute ventilation tend to increase when breathing through a mouthpiece [28, 29]. To show to what extent dead volume can vary during uncontrolled breathing through a device, dead space estimations obtained during a 5 min measurement session by a spirometer equipped with a multi-gas analyzer (MSX, Ferraris Cardiorespiratory) are reported (figure 2); values range from 140 to 210 ml, which means that breath-by-breath calculations have to be performed for sampling on multiple breaths.

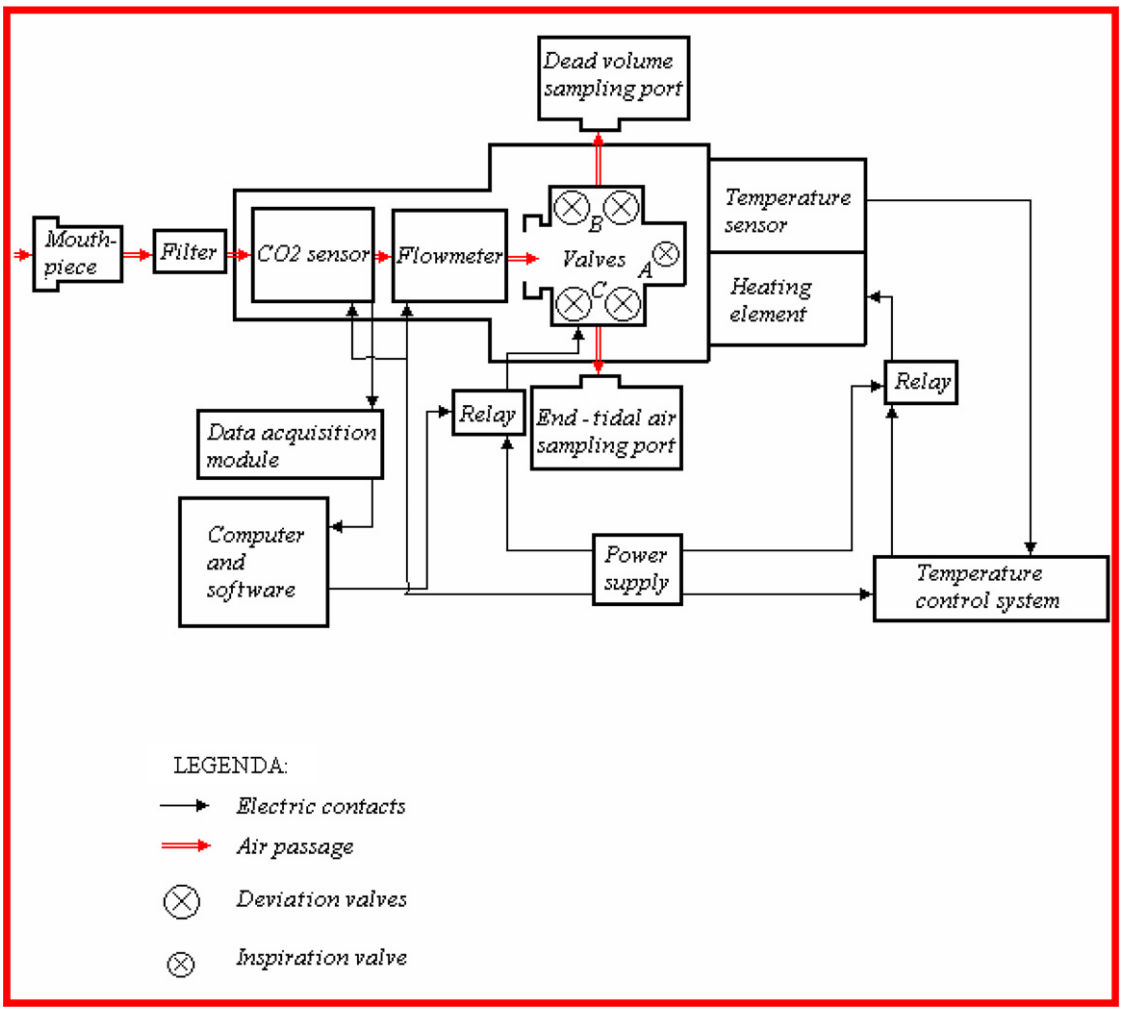

Figure 3. Block diagram of the breath sampler: hydraulic and electric connections are represented by arrows and connectors, respectively. At any time, a single group of valves is open: valve *A* during inspiration, valves *B* or *C* during expiration corresponding to the exhalation of dead volume and end-tidal air respectively.

3.2. System description

A block diagram of our alveolar air sampler is reported in figure 3. The study subject blows into the device through a disposable mouthpiece and a low pressure drop bacterial filter (MGCON-FIL 495, Morgan Italia). The flow measurement is performed by a microbridge mass airflow sensor (AWM5104VN, Honeywell) with a Venturi-type flow housing. It measures flows up to 20 standard liters per minute with a good repeatability (0.5%) and a limited pressure drop. Flow measurements are performed only when the subject is expiring; an analog output below the expected range (1–5 V) indicates that flow direction is reversed during inspiration.

The carbon dioxide concentration is measured by wavelength modulation spectroscopy (WMS), a very effective optical absorption technique for the detection of gases. The emission wavelength of a temperature-stabilized laser diode is modulated, by varying the injection current, to scan a single absorption peak of the target gas with a high resolution. In our device, a wavelength of 2004 nm was selected to monitor carbon dioxide, with a scan range of 1 nm. As light propagates through the sample, the wavelength modulation is converted to an amplitude modulation by the different absorptions of the target species at different points of the absorption peak. A second modulation, at a higher frequency, is applied in order to perform lock-in demodulation, so that the output signal of the photo-detector preamplifier contains ac components at the modulation frequency and its harmonics. The second harmonic was selected by doubling the frequency of the reference signal used to synchronize a lock-in amplifier (demodulation). In theory, if the amplitude of the modulation range is small compared to the line width, the spectrum of the *n*th harmonic is proportional to the *n*th derivative of the absorption line spectrum that can thus be calculated [30]. The concentration value is then derived, thanks to the well-known Lambert–Beer law. A laser diode (VCSEL 2004 nm, Vertilas) is used to generate the beam for the carbon dioxide measurement, which is sent through the airflow sensor so that airflow and concentration can be measured together (figure 4). A multifunction data acquisition module (USB 6210, National Instruments) is used to acquire data from both the flow sensor and the lock-in amplifier, and to control the valves directing the sample flow. A solenoid valve (*A*) (solenoids series 44 A, RS), located on the opposite side of the mouthpiece, allows

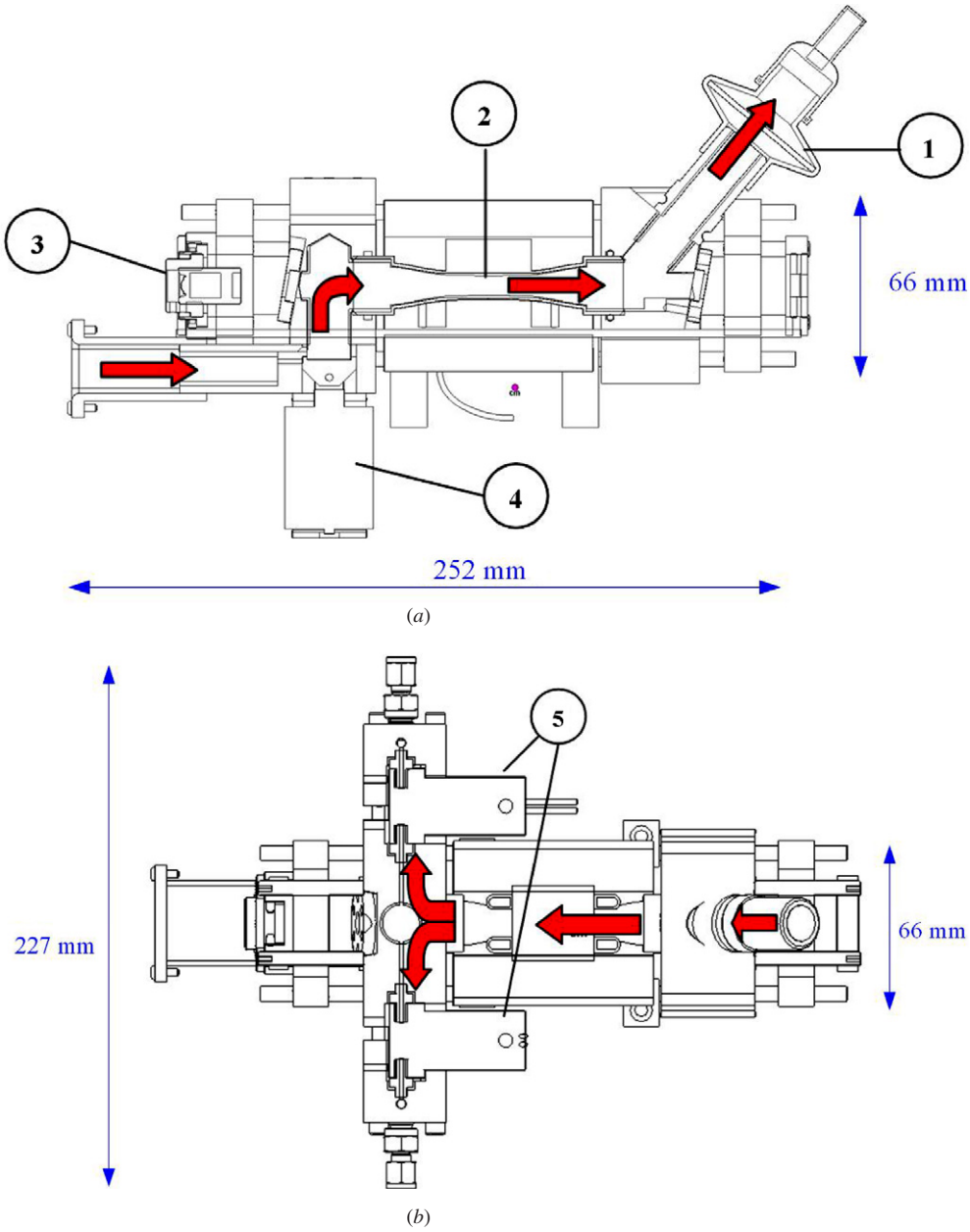

Figure 4. The breath sampler. Air and sample flow are shown during inspiration (*a*) and expiration (*b*). The main system components are also shown: (1) bacterial filter, (2) flow sensor, (3) diode laser, (4) ambient air inlet valve, (5) sampling ports.

the ambient air into the device during inspiration, while two pairs (*B*, *C*) of fast solenoid valves (100T2, Bio-Chem Valve™ Inc., reaction time 20 ms), located on both sides of the device, control the sampling ports for the collection of dead space and end-tidal air (figure 3). The use of a pair of valves is necessary to decrease head losses, since inert valves with PTFE body, short actuation time and sufficiently large bore size are not commercially available. During operation, valves *A*, *B* and *C* are opened in sequence corresponding to inspiration, collection of dead volume and end-tidal air respectively. The whole system is thermostated at 40 °C by means of a thermocouple, cable and band heaters and a microprocessor controller in order to avoid any condensation.

Dedicated software was developed in a Labview™ environment to provide users with a graphical interface so that they can calibrate the flow and carbon dioxide sensors, visualize and save the data (carbon dioxide concentration, expiration flow and volume values), and choose from the various sampling modes. In fact, if the reversal of flow direction triggers the closure of valve *C* and the simultaneous opening of valve *A* at the beginning of inspiration, as well as the closure of valve *A* and the simultaneous opening of valve *B* at the beginning of expiration, three options are available to trigger the closure of the dead space sampling port and the simultaneous opening of the end-tidal air sampling port: (1) attainment of a set-point value for the absolute concentration of CO_2, (2) attainment of a user-defined percentage of the end-tidal CO_2 concentration (recorded in the last respiration cycle) and (3) attainment of Fowler's

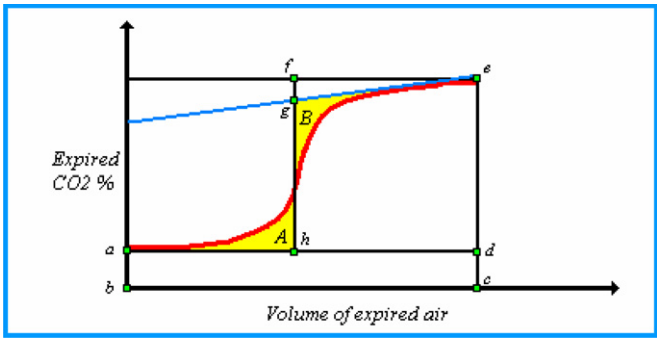

Figure 5. Details of the algorithm for the implementation of Fowler's method. The difference between the areas of the shaded regions *A* and *B* is calculated by (1) subtracting the area of the baseline rectangle *abcd* from the area of the region *abce* below the curve of CO_2 concentration versus expired volume and (2) subtracting the area of the rectangle *hdef* and adding the area of the triangle *gef* to the previous result.

dead space volume (calculated from data relevant to the last respiration cycle).

Fowler's manual estimation of anatomic dead space relies on a tedious graphical procedure prone to inaccuracies and errors. Several simplified approaches have been proposed to obtain easier determinations [31, 32], but such approximations deliver quantities that differ from Fowler's dead space by as much as 10% or more [32]. More recently, an algorithm was proposed for use on real-time computer-assisted determinations [33], but this approach turned out to be too time consuming for breath-by-breath calculations. We developed an algorithm that implements Fowler's method in just five steps (figure 5): (i) construction of the CO_2 concentration versus expired volume curve from raw data; (ii) zeroing of the curve by subtraction of the constant baseline value; (iii) fitting of two fourth-order polynomials to obtain an analytical expression of the curve; (iv) drawing of the tangent with slope ε (parameter to be set by the user) and calculation of the parameters included in the analytical expression of the function

$$F(V_D) = A(V_D) - B(V_D), \qquad (4)$$

where V_D is the dead volume, $A(V_D)$ and $B(V_D)$ are the areas of the shaded regions *A* and *B*; (v) iterative determination of the dead volume V_D as the zero of $F(V_D)$. Working on the analytical expression of the function $F(V_D)$ representing the difference between the values of the area of regions *A* and *B* is critical for speed, and finding the zeros of a function is a common task in computer-assisted calculations.

The whole procedure very much resembles Fowler's manual estimation with the graphical approach. In this case, the human assessor draws a straight line fitting the plateau of the curve of CO_2 concentration versus expired volume; then he/she moves back and forth the vertical line that limits the shaded regions *A* and *B* until they have the same area (figure 5). If the graph is plotted on a graph paper, it means that the same number of squares have to be framed in the *A* and *B* regions. There is a margin of arbitrariness in the drawing of the straight line, which introduces a spread in results obtained by different assessors (or even by the same assessor in repeated estimates). Real curves of CO_2 concentration versus expired volume do not show an actual plateau, but CO_2 concentration continues to increase slightly with the volume until the end of expiration and it is not that obvious where the plateau begins. Fortunately, the dependence of dead space estimate on the slope of this

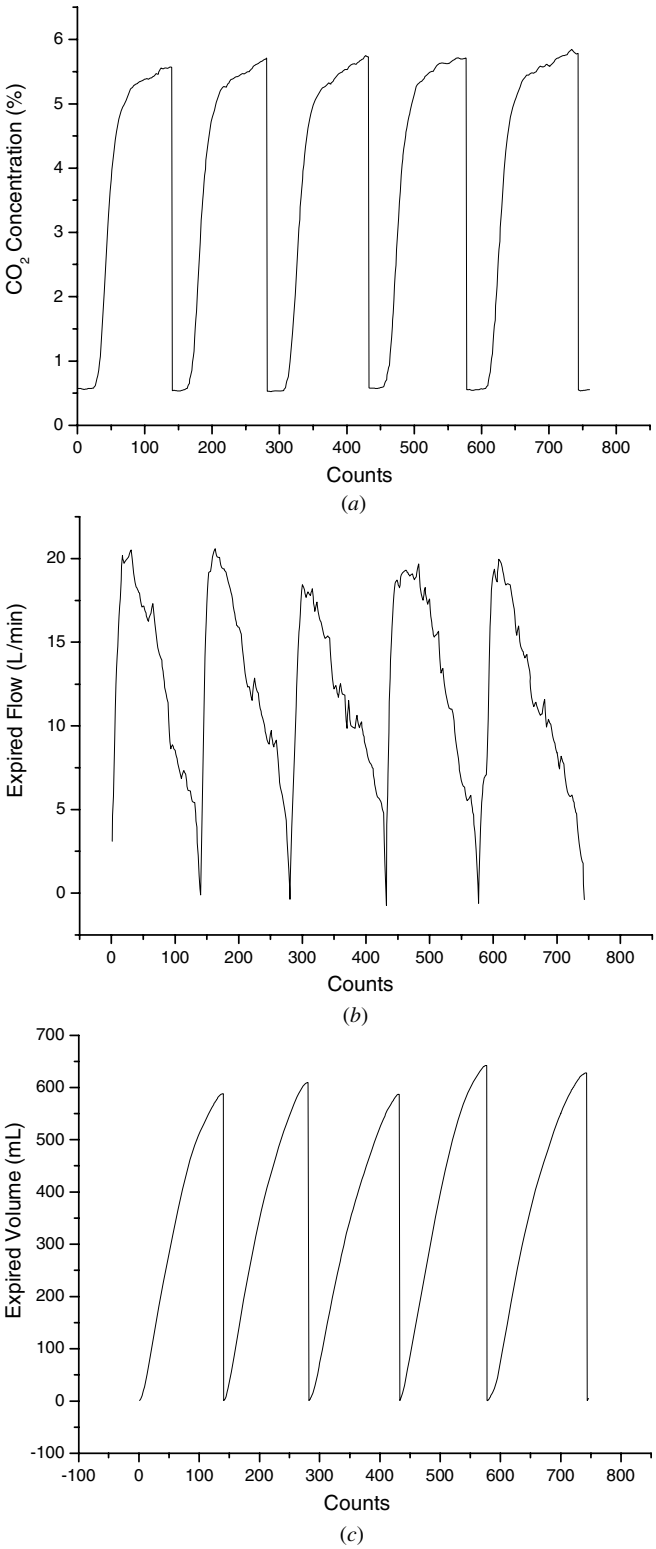

Figure 6. Carbon dioxide concentration (*a*), expired flow (*b*) and volume (*c*) measured in a series of repeated breaths. The sampling rate is 5 Hz; data regarding expiration flow and volume are only acquired during expiration.

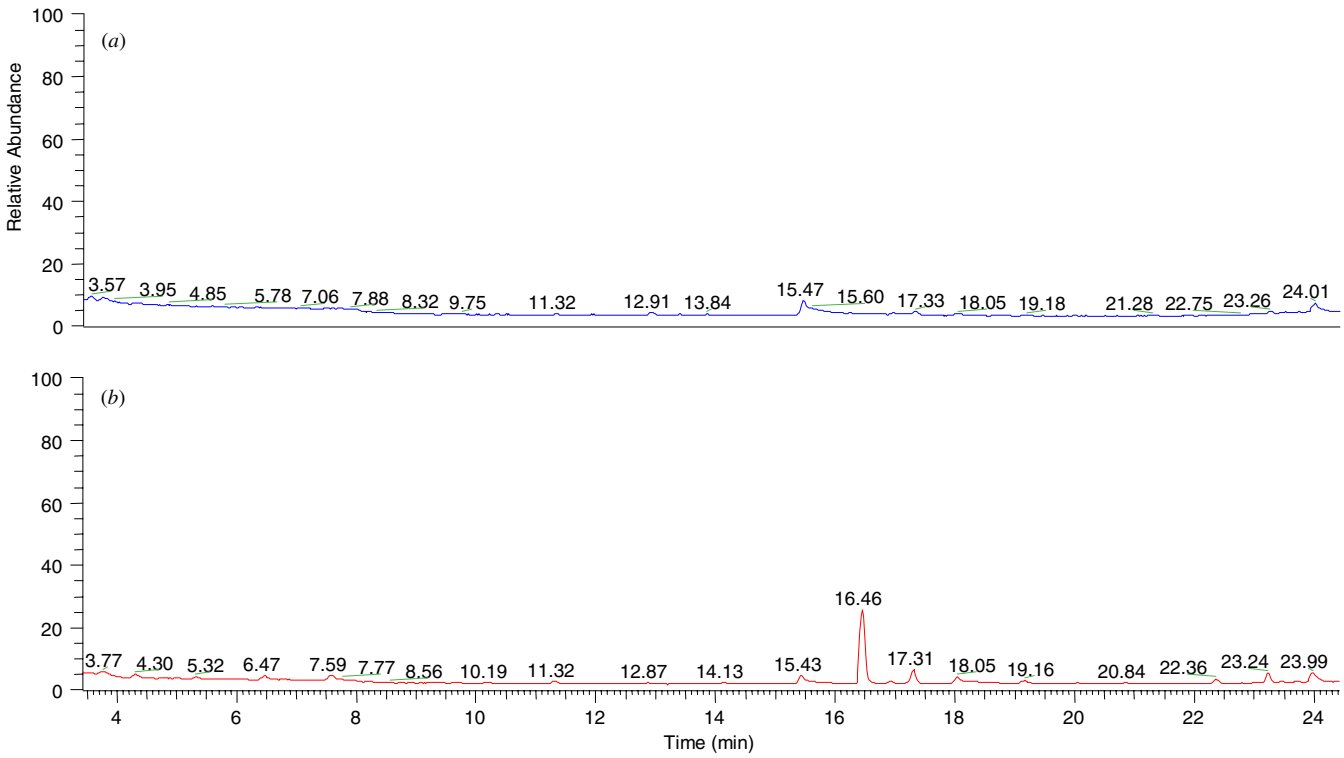

Figure 7. Results of the contamination test: chromatograms of reference air (*a*) and air passed through the air sampler and collected by a Nalophan™ bag (*b*) show almost no difference, demonstrating that system components do not contaminate the sample.

Table 1. Comparison of anatomical dead space estimations in data of single breaths obtained from five test subjects: proposed algorithm, graphical method (average ± standard deviation), absolute and percentage deviations. The standard deviation accounts for the variability due to manual estimations of four assessors on the same data.

Subject ID	Dead space volume (ml)		Absolute deviation (ml)	Percentage deviation (%)
	Algorithm	Manual		
1	232	237 ± 8.5	5	2
2	144	140 ± 3.5	−4	3
3	151	140 ± 3.5	−11	8
4	121	125 ± 5	4	3
5	179	170 ± 8	−9	5

straight line is not critical, and the spread of results is limited to a few per cent (table 1).

3.3. Testing the system

The flow sensor was calibrated by using a primary flow calibrator (MiniBUCK M-30, A P BUCK), while the CO_2 sensor was calibrated with a certified mixture of carbon dioxide in nitrogen (5% v/v). The subject needs to blow the equivalent of 23 ml of water (about 18 mmHg) for the head losses of the system to be overcome. The profiles of CO_2 concentration, expired flow and volume versus time in a series of repeated breaths are reported in figure 6. As can be seen, the attainment of a rather regular breathing pattern is possible with the device. The alteration in the respiratory parameters due to the interaction with the measurement system has been reported [29], and the hypothesis of a shift in control from the automatic respiration centers to the cerebral cortex has been made [34]. The reproducibility of breath sample collection can be improved by giving subjects advice on how to regulate voluntary breathing [23]. An upgraded version of the user interface is thus currently under development which will enable subjects to synchronize respiration to a metronomic signal and to maintain a constant tidal volume in different respiration cycles, thanks to a visual indication of the expired volume.

Possible contamination of the sample by the device was tested by comparing the composition of reference air and reference air passed through the sampler and collected in a Nalophan bag (figure 7). The only difference in the two chromatograms obtained by pre-concentration in an absorption tube, and thermal desorption followed by GC/MS analysis, consists in a peak relevant to 2-methyl-1,3-dioxolane, which is a contaminant released by the bag. This demonstrates that no compound is released that can be detected by the above technique.

The algorithm used for the automatic calculation of the anatomical dead space was tested by comparing its results on breath records from five test subjects with results of manual graphical evaluations carried out by four assessors. Data reported in table 1 show that variability in the manual graphical determinations is comparable to the deviations between the algorithm and the average of manual graphical results. The automated system thus basically acts as an additional human assessor in the panel.

4. Discussion

Breath analysis is fascinating due to the simplicity of the idea and the elegance of the method. Its approach to patients in a completely non-invasive way and its promise of guaranteeing real-time results are truly avant-garde. However, despite the efforts of scientists for over 30 years and the current availability of sensitive analytical devices, there is still a significant difference between theory and practice. In our opinion, two main reasons are behind this difference. The first one is the insufficient understanding of medical and physiological aspects, which leads to following not well-defined goals and prevents the choice and the characterization of the right type of patients and control subjects. The second is the lack of reliable sampling procedures, which increases variability, alters multivariate data patterns and hinders the comparison of results obtained by different groups. Our aim was to help to develop a standardized sampling protocol. The collection of multiple breaths should allow samples to be obtained that are more representative of a subject's condition. The separation of dead space and end-tidal fraction should provide important data on the origin of compounds, which can then be used to discriminate between endogenous and exogenous chemicals. The availability of large volumes of sample allows pre-concentration and enhances the possibility of detecting species that are only present in breath at a trace level.

However, the system itself does not guarantee better results, and an extended validation is needed to develop a reliable collection protocol capable of avoiding artifacts due, for example, to the psychological aspects of the human–machine interaction. The choice of developing a CO_2 sensor based on WMS offers both short- and long-term advantages. A short response time is achievable which is needed to provide data, perform calculations, estimate dead volume and control the valves quickly enough to sample dead space and end-tidal air on multiple breaths. Furthermore, the shift in frequency of the absorption measurements from values near dc to high frequencies makes WMS far more sensitive compared to ordinary optical absorption techniques. This is not fundamental for CO_2 but is of the utmost importance for monitoring marker compounds that are present in breath at ppb or even at ppt levels. The advances in the field of diode and quantum cascade lasers, which are tunable in a wide range of wavelengths, are expanding the offer of new devices and decreasing costs. These results lay the foundation for the development of portable diagnostic instruments as soon as new breath markers will be discovered [35].

5. Conclusions

There are many advantages of separately collecting different breath fractions on multiple breaths: higher sample volumes, insights regarding the origin of compounds and increased reproducibility of sampling. The dead space volume varies significantly among subjects and, in the same subject, on the basis of sampling conditions. For these reasons, a breath-by-breath estimate of dead volume is needed. However, the short time available for data acquisition and treatment on a single breath and the demanding constraints to ensure comfort and safety to the subject under test make the development of the breath sampler a challenging task. In this work, the design, realization and testing of a CO_2-triggered breath sampler capable of separate collection of dead space and end-tidal air has been presented and discussed and its capability of separate collection of dead space volume and end-tidal air demonstrated.

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