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A dual mode breath sampler for the collection of the end-tidal and dead space fractions

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ABSTRACT

This work presents a breath sampler prototype automatically collecting end-tidal (single and multiple breaths) or dead space air fractions (multiple breaths). This result is achieved by real time measurements of the CO_2 partial pressure and airflow during the expiratory and inspiratory phases. Suitable algorithms, used to control a solenoid valve, guarantee that a Nalophan® bag is filled with the selected breath fraction even if the subject under test hyperventilates. The breath sampler has low pressure drop (<0.5 kPa) and uses inert or disposable components to avoid bacteriological risk for the patients and contamination of the breath samples. A fully customisable software interface allows a real time control of the hardware and software status. The performances of the breath sampler were evaluated by comparing (a) the CO_2 partial pressure calculated during the sampling with the CO_2 pressure measured off-line within the Nalophan® bag; (b) the concentrations of four selected volatile organic compounds in dead space, end-tidal and mixed breath fractions.

Results showed negligible deviations between calculated and off-line CO_2 pressure values and the distributions of the selected compounds into dead space, end-tidal and mixed breath fractions were in agreement with their chemical–physical properties.

1. Introduction

Exhaled air is a complex mixture of inorganic gases such as nitric oxide (NO) and carbon monoxide (CO), volatile organic compounds (VOCs) like acetone and isoprene and low or non-volatile compounds like hydrogen peroxide or cytokines solubilized in breath aerosol [1].

Several studies have investigated the possibility of using breath analysis for diagnostic purposes, suggesting correlations between health conditions and breath concentrations of chemical markers [2]. Largely cited examples of such correlations are acetone and diabetes mellitus, ammonia and dysfunctions in protein metabolism, dimethylamine and renal diseases, dimethyl sulphide and hepatic dysfunctions, hydrocarbons and abnormal lipid peroxidation [3–5]. Breath analysis seems a promising diagnostic tool both for the screening of patients affected with different kinds of diseases and the monitoring of physiological processes. The very low invasiveness of sample collection and the possibility to monitor in real time the concentrations of breath markers, are the main potential advantages of this approach, which can be also adopted for children or patients in critical conditions.

Although the potential applications in health care are enormous, breath analysis is still a challenge [6] due to the high cost and need of skilled personnel for the analytical instrumentation employed in breath profiling, typically gas chromatography with mass spectrometry (GC–MS), ion mobility spectrometry (IMS), proton transfer reaction mass spectrometry (PTR-MS) or selected ion flow tube mass spectrometry (SIFT-MS). Nevertheless, some breath tests are currently used in the clinical practice [5]. For example, urea breath test is used to identify infections by *Helicobacter pylori*, a bacterium associated to duodenal and gastric ulcers, stomach cancer and non-ulcer dyspepsia [7–9]. In such test, the patient is administered ^{13}C or ^{14}C labelled urea that, in case of infection, is split into ammonia and labelled carbon dioxide by *H. pylori*'s urease. The labelled carbon dioxide is then eliminated with exhaled breath. The Heliprobe System® (Kibion, Sweden) is a commercially available platform for urea breath test that does not require the use of expensive and sophisticated instrumentation to identify the presence of *H. pylori*.

Another critical aspect hampering the progress of breath analysis is the lack of standardized procedures for the breath tests, although general guidelines for controlled breath sampling and analysis can be followed [10,11]. In this regard, several breath sampler

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prototypes have been proposed in literature. A breath collection device was described by Cope et al. [12]. The subject breathed through a mouthpiece equipped with an antibacterial filter connected to a non-rebreathing valve, while two transducers constantly monitored pressure and volume of the exhaled air. An infrared sensor placed after the non-rebreathing valve measured the partial pressure of CO_2. Breath flowed through a relatively large duct that minimized pressure drops and served as a reservoir before the sample reached the external environment. The signals from the transducers and the CO_2 sensor were displayed on a personal computer screen to provide constant feedback to the operator and the patient. A pump allowed the exhaled breath to be drawn through a flow divider and two duplicate thermal desorption tubes. In this configuration, the instrumental dead volume was 70 mL. Another breath sampler was developed by Miekisch et al. and consisted of a disposable mouthpiece, a series of polyethylene T-pieces and a CO_2 infrared sensor [10]. Exhaled air could be sampled by either a gas-tight syringe or a Tedlar bag connected just before the CO_2 sensor. The real time capnogram displayed on the screen allowed the selective collection of end-tidal air. A drawback of this device was that air sampling had to be manually performed or triggered by an operator who looked at the capnogram, which limited reproducibility. The use of pre-evacuated stainless steel canisters opened by the subject under investigation himself is also reported in Lindstrom [13].

A further breath sampler allowing the collection of large volumes on multiple breaths was proposed [14]. The subject breathed through a mouthpiece and breath passed through a CO_2 infrared sensor based on laser spectroscopy and a flow meter. A dedicated software acquired the respiratory parameters from both the CO_2 sensor and the flow meter to control a system of solenoid valves. This device automatically selected the end-tidal air fraction by either the Bohr's [15] or Fowler's [16] method. Breath was collected in Nalophan® bags and analysed by thermal desorption gas chromatography–mass spectrometry (TD-GC–MS). Although end-tidal gas could be sampled correctly, this system had a few weak points: (1) thermal stress and insufficient mechanical stability caused the loss of alignment of the optical system used for CO_2 measurements, which needed frequent calibrations; (2) a relatively high pressure drop (2.3 kPa against a desirable target lower than 0.5 kPa) due to the small orifices of valves and connections to the Nalophan® bags; (3) poor bacteriological safety, as the internal ducts were hardly accessible for cleaning and sterilization and (4) presence of a large instrumental dead volume (about 50 mL).

The breath sampler presented in this work is inspired by Cope's system but allows for (i) automatic sampling over end-tidal (single and multiple breaths) or dead space air fractions (multiple breaths) and (ii) selection of air coming either from the upper airways (dead space sampling) or from the lungs (end-tidal sampling).

2. Materials and methods

2.1. Hardware

The system was designed with a specific attention to the subject's safety and comfort as well as to a controlled and reproducible sampling of selected breath fractions (end-tidal or dead space). In particular, the following requirements were defined: (i) components compliant with medical use; (ii) bacteriological safety; (iii) overall pressure drop less than 0.5 kPa; (iv) negligible contamination of samples; (v) instrumental dead volume much lower than the minimum sampled volume of breath; (vi) real time automatic adaptation to variations of the subject's breathing pattern.

The schematic diagram of the breath sampler is shown in Fig. 1 and consists of two sections. The analysis section, which measures airflow, pressure and CO_2, is in contact with the subject through a sterile and disposable mouthpiece. The sampling section, where breath is sampled and collected into disposable Nalophan® bags

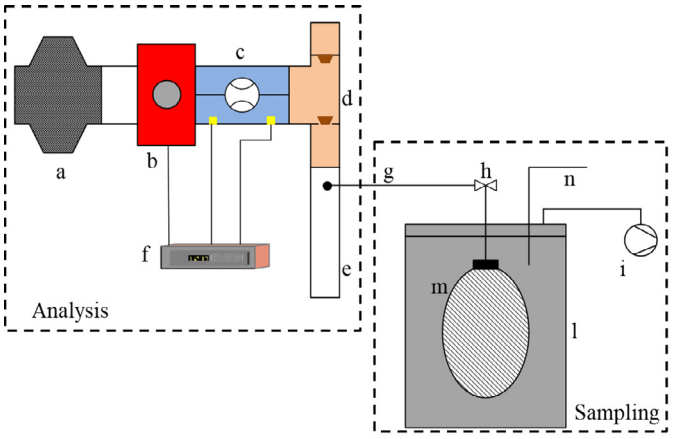

Fig. 1. Schematic diagram of the breath sampler. (a) mouthpiece and anti-bacterial filter; (b) CO_2 sensor; (c) flow meter (pneumotachometer); (d) non-rebreathing valve; (e) collection chamber of exhaled breath; (f) Mercury module; (g) connection tube; (h) solenoid valve; (i) vacuum pump; (l) airtight container; (m) Nalophan® bag; (n) tube controlling the pressure inside the container.

(PET, polyethylene terephthalate film, thickness 20 μm supplied by Kalle GmbH, Germany), is connected to the analysis section [17,18]. The Nalophan® bag can be replaced with a different bag, according to the specific analyte (e.g. ammonia) to be analysed, like for example a Tedlar bag [19,20]. All the breath sampler components in contact with breath are of inert material, such as polypropylene, polyethylene and Teflon, and are kept at 40 °C by an insulated electric wire (resistance = 3.5 Ω/m) to avoid condensation. To reduce the possibility of bacteria and virus cross-contamination, the subject breathes through a sterile mouthpiece connected to an antibacterial filter (Microgard IIB by Carefusion, Italy). A graphical interface, developed in LabVIEW® (version 7.1, National Instruments, United States), shows real time values of CO_2, airflow, pressure and volume of exhaled air. In compliance with the standard ISO 9241 on the ergonomics of human–computer interaction, the software interface not only provides all data regarding the hardware status, but it is also fully customi sable. The output file is in Excel® format. The operator can at any time visualize the status of the system, modify the settings and save all the information regarding the patient and the sampling mode.

A fast mainstream sensor with a response time lower than 60 ms (Capnostat® 5, Respironics Inc., Philips, Italy) measures the CO_2 partial pressure (kPa) and the respiration rate (breaths per minute). Capnostat® 5 requires a supply voltage of 5 V and is equipped with RS-232 interface to communicate with the Mercury module (Respironics Inc., Philips, Italy). The Mercury module (7.62 cm × 9.78 cm × 2.73 cm, 5 V, RS-232 interface) acquires data from the Capnostat® 5 and measures the airflow and pressure by a pneumotachometer characterized by a low pressure drop (0.21 kPa at 60 L/min).

The gauge pressure transducer is located in the same module housing an absolute pressure transducer (Respironics Flow Meter Series 3, Philips, Italy) for the measurement of barometric pressure. The disposable airway adapters are in inert material (Respironics, paediatric/adult single patient use airway adapter, 5.7 cm × 2.2 cm, Philips, Italy). airflow (L/min), pressure (kPa), volume (mL) and CO_2 (kPa) values are transmitted from the Mercury module to a computer in real time. The Mercury module samples airflow and airway pressure at 200 Hz, whereas Capnostat® 5 samples CO_2 at 100 Hz.

A sterilisable, non-rebreathing two-way, three-port valve (model 1410, 10 cm × 5.7 cm × 3.7 cm, 22 mm outer diameter, 15 mm internal diameter, Hans Rudolph Inc., United States,) located after the sensors allow the subject to inhale and exhale with negligible effort. Two of the ports integrate a flexible silicone diaphragm that opens or closes according to the air pressure so that only unidirectional flow is possible. The third port is connected to the mouthpiece through the