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# Measure chain for exhaled breath collection and analysis: A novel approach suitable for frail respiratory patients

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ABSTRACT

Exhaled breath analysis, a non-invasive diagnostic procedure, is based upon a sampling device for breath collection, an apparatus for the sample delivery into the measure chamber and a gas sensor array. Here a novel approach for the design and realization of these three components is presented. The volatile mixture composing the exhaled breath is entrapped onto an adsorbing cartridge via an innovative device able to collect exhaled breath from an individual normally breathing into a mouthpiece for three minutes. This procedure is very simple and cartridges outperform sampling bags in terms of preservation and transportability. Moreover, a thermal desorption process of the volatiles adsorbed on the cartridge can partially separate them by a given temperature profile. This separation, performed by the desorbing apparatus, improves sensor resolution via a preconcentration strategy. This method improves sensor resolution. The gas sensor array employed in this work is composed by a set of eight Quartz Micro Balances (QMB) coated by eight different anthocyanins. The Pneumopipe, the innovative breath collecting device, is described and tested in a proof of concept study involving all the measure chain: two small groups of Chronic Obstructive Pulmonary Disease (COPD) patients and control individuals have been perfectly discriminated and repeated measures on the same individuals showed an optimal reproducibility as verified by calculation of the relative standard deviation.



## 1. Introduction

Exhaled breath finger-printing, in spite of its minimal invasivity, is not still employed in the clinical practice mainly due to the lack of standard procedures [1]. This work aims to respond to some of the key questions regarding breath analysis standardization [1,2]. The core innovation regards the exhaled breath sample. Exhaled breath should be collected via a suitable interface with the individual and, then, the collected exhaled breath sample has to be delivered into the measure cell via an ad hoc interface with the measuring instrument. The design and realization of the interface impacts each step of the measure chain; thus, the whole measure chain will be the object of this paper.

Exhaled breath collection asks for reproducible and standardized techniques in order to be representative and effective [3]. Direct (subject directly breathing into the measuring system without any intermediate steps) or indirect methodologies exist. The latter requires the exhalate sample to be stored in an adequate medium to be analyzed later.

Indirect sampling can be performed via bags of Tedlar or other low-emission plastic materials, glass vials, stainless steel containers and cartridges containing adsorbent substances (sorbent traps). Tedlar bags and cartridges of absorbent material are the most commonly used media.

Collection of exhaled breath on adsorbent cartridges allows sample pre-concentration to increase the resolution power of the instrument (typically round tens of ppb). Moreover sample

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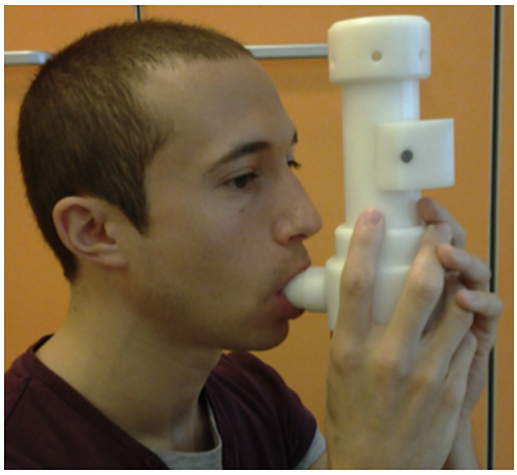

**Fig. 1.** Individual performing breath collection via pneumopipe.

adsorbed in a cartridge can be easily stored and transported. However, adsorbent cartridges offer a high resistance to air transit, thus a dedicated pneumatic device must 'help' the subject pushing exhaled air into them. All the methods realizing these operations are based on complex procedures or/and awkward systems [4–8].

Exhaled breath collection on adsorbing cartridge could be also realized via two sequential indirect operations: first the subject fills the bag, and then bag content fills the cartridge. This latter method, in spite of its simplicity, has low reproducibility and it is not fully reliable.

The solution here proposed is articulated in the description of novel contributions in the three main steps of the measure chain: a device for exhaled breath sampling directly on cartridges (named Pneumopipe), an apparatus for cartridge thermal desorption into a measure-cell (named Breathstiller) and a gas sensor array based on Quartz Microbalance functionalized by anthocyanins (named Bionote). The Pneumopipe has been designed and fabricated by the authors (EU patent pending [9]). The main features of this device are the simplicity of the sampling procedure (also feasible for respiratory impaired subjects), and the transportability of the collected samples (usually not applicable to a sampling bag). The Pneumopipe designed by the authors at the University Campus Bio-medico allows the exhaled breath collection from an individual normally breathing through a mouthpiece, catching the volatile compounds on an adsorbing cartridge (see Fig. 1).

The sampling procedure and the interface for the following desorption of the sample must be suitable for different instruments. In fact, breath-print consists of exhaled breath measurement and translation in a numerical pattern [10] and this pattern can be obtained by:

(a) analytical chemistry instruments (Gas Chromatography–Mass Spectrometry (GC-MS), Proton Transfer Reaction–MS (PTR-MS), Selected Ion Flow Tube–MS (SIFT-MS)) [11–14];
(b) direct non-selective fingerprinting techniques [15].

The latters are usually performed by gas sensor arrays designed as artificial olfactory systems [16–18]. Gas sensor array output responses consist of a pattern (breath-print) characteristic of the individual and of his/her health conditions. The breathprints registered in several experiments showed evidences of correlation with certain diseases: lung cancer [19–21], Chronic Obstructive Pulmonary Diseases (COPD) [22–24], asthma [22,25], just to mention the main respiratory diseases investigated among many others.

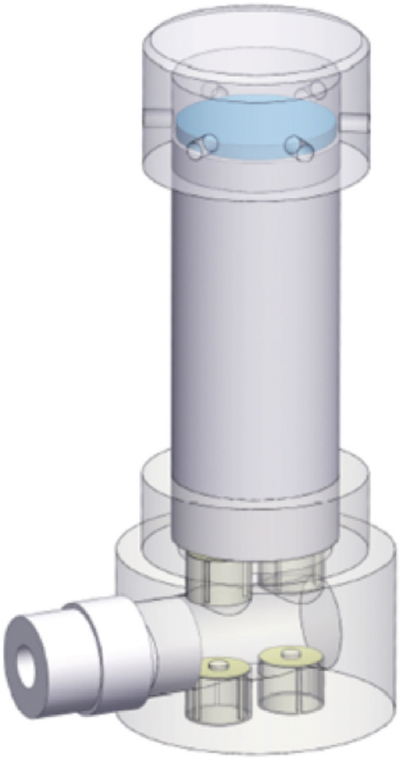

**Fig. 2.** General overview of the Pneumopipe.

Regarding gas sensor arrays, thermal desorption of cartridge content into the measure cell is not a routine operation as for the analytical chemistry instrument. This work describes the use of the breathstiller (see Fig. 2) designed, fabricated and tested by the authors, for the thermal desorption of cartridge directly into the gas sensor array measure cell.

## 2. Materials and methods

### 2.1. Pneumopipe

The Pneumopipe allows collecting exhaled breath into an adsorbent cartridge, overcoming the drawbacks mentioned above with reference to the state of the art.

The Pneumopipe (see Fig. 2 for a general overview), thanks to its double-chamber structure (Fig. 3), permits a continuous sampling of exhaled air, even during an inhalation step. Fig. 3 shows that the lower chamber of the device is the first encountered by the exhalation which is then directed into the upper chamber by opening valve number 2 as effect of the exhalation act. During this phase (denoted with (a) in Fig. 3) the influence by environmental air is prevented by the valve number 1. Valve number 3, normally opened by the patient exhaling, avoids the perceiving of a resistance to respiration by the individual performing the exhalate collection. In the second phase, during the inspiration act, valves 3 and 2 are normally closed, isolating the exhaled breath collected in the previous phase. This, prevents the contamination of the exhaled breath by the environmental air entering through valve number 1, opened by the inspitration act itself, and minimizing the resistance to respiration. During phases (a) and (b) the sampling of the exhaled breath is operated by a pump, which sucks the exhaled breath collected in the main chamber of the pneumopipe at a constant flow of 80 ml/min for 3 min. The pump delivers the exhaled breath into an adsorbent cartridge located before the pump itself.