Alveolar Air Volatile Organic Compound Extractor for Clinical Breath Sampling

Geethanga de Silva and Fred R. Beyette Jr.

Abstract— **Alveolar air Volatile Organic Compound (VOC) extractor is a handheld breath-sampling device for clinical breath analysis. The device consists two main components: (1) An alveolar air separator, (2) A VOC extractor. The alveolar air separator splits exhaled air based on total exhaled air volume directing alveolar air towards the VOC extractor and dead space air to into an exhaust channel. The VOC extractor collects the VOCs from alveolar air into a modified Sold Phase Micro Extraction (SPME) filament. Feasibility of using the SPME filament to collect a quantifiable breath sample directly from exhaled breath is experimentally validated. Exhaled breath acetone is quantified using alveolar air VOC extractor and a GC/MS system.**

I. Introduction

More than one thousand Volatile Organic Compounds (VOCs) have been detected in exhaled breath including some that have been identified as biomarkers for various diseases and metabolic conditions [1]. These biomarkers are associated with lipid peroxidation, liver diseases, renal failure, allograft rejection, cancer, glucose metabolism, cholesterol metabolism and many other health conditions. Although breath analysis inherently has many advantages, it still remains underutilized as a standard diagnostic method. This is partly due to inadequacy of medical devices needed for clinical breath analysis. Breath sampling is a critical step in breath analysis. To this date there is no breath-sampling device suitable for routine clinical diagnosis [2], [3].

Alveolar breath sampling is more suitable than mixed expiratory sampling in producing a standard breath sample. Mixed expiratory sampling collects the total volume of air exhaled, including the air in the upper regions (nose, pharynx, trachea and bronchi) of the respiratory systems (so called dead space air) that does not participate the in the gas exchange [4]. Alveolar air located in the alveoli, alveolar sacs and atria, participates in the gas exchange and contains high endogenous VOC concentrations. Inclusion of dead space air in a breath sample leads to variable dilution of the breath sample and contamination from exogenous VOCs [5]. Most of the alveolar breath-sampling devices are based on the Haldane-Priestly tube [6]. Devices that adapted this method empty the total exhaled air into a tube or a temporary reservoir and alveolar air is pumped out to a breath sampling container [7], [8], [9]. Although these devices serve the purpose of collecting alveolar air, they lack portability, ability of collecting a standard sample and ease of use as a standalone Point-of-Care (POC) device.

Concentrations of identified biomarkers fall in the nmol/l to pmol/l (ppb-ppt) range and usually need pre-concentration prior to analysis. Common methods of pre-concentration are adsorption onto sorbent traps, coated fibers and cryofocussation. Extracted VOCs are transferred from the adsorption material into an analytical device by heating or microwave energy. Breath samples are analyzed using high sensitive instruments such as Mass Spectrometry (MS) systems. MS systems are often coupled with Gas Chromatography (GC) for initial separation [2], [3]. Complexity of MS based systems has lead to the development of sensor-based analyzers for rapid POC diagnosis. Sensor based VOC analyzers may have a promising future, but at the current state they are lacking the required sensitivity and selectivity for direct breath analysis. VOC sensors are highly sensitive to flow rate, humidity and temperature which varies dynamically in exhaled breath and none of the sensors have demonstrated the feasibility of directly analyzing exhaled breath. Most of the sensors do not have the required response time to perform breath-to-breath analysis.

Although development of sensor based portable VOC analyzers are essential to realize the true potential of breath analysis, the current state of the art demands for a clinically viable device capable of collecting a breath sample at POC into a GC/MS compatible container. Hence, we introduce a portable device that extracts VOCs from alveolar air into a compact container. Sample container is a polymer filament inside a metal needle and can be analyzed directly using GC/MS system without further sample preparation. The device presented in this paper mainly consists of two parts: (1) An alveolar air separator, (2) A VOC extractor. The alveolar air separator identifies when alveolar air is expired based on the volume of air expired and directs the alveolar air towards the VOC extractor [10]. VOC extractor collects the VOCs in alveolar air into a modified Solid Phase Micro Extraction (SPME) filament (65µm PDMS/DVB, fused silica, 24 Ga, Supelco). SPME filament can be removed from the device and inserted into a GC/MS system for analysis.

Geethanga de Silva (phone: 514-461-4415 e-mail: desilvga@mail.uc.edu) and Fred R. Beyette Jr. (e-mail: beyettfr@ucmail.uc.edu) are with the Department of Electrical Engineering and Computing Systems, University of Cincinnati, OH, 45220 USA.

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II. ALVEOLAR AIR SEPARATOR

Alveolar air separator diverts dead space air to an exhaust path and directs alveolar air into alveolar air channel [10]. The device consists a Fleisch type flow meter, a flow diverting gate as shown in Fig. 1. The flow diverting gate is mounted on a plunger of a pull type linear solenoid actuator. When the actuator is energized, the flow diverting gate is in the position shown in Fig. 1 (a). During this stage exhaled air is diverted to an exhaust path. Once the actuator is de-energized, the flow diverting gate moves into the mouthpiece and directs exhaled air through the alveolar air channel as shown in Fig. 1 (b). Dimensions for the exhaled flow measuring mouthpiece are determined by a study conducted to evaluate the user comfort level of flow meters that require breathing for an extended period of time [11].

A. Flow meter calibration

Flow meter of the alveolar air separator is calibrated using a commercial spirometer (Minispir, Medical International Research USA, Inc.). The flow meter and the commercial spirometer are serially connected forming a single channel. Nitrogen gas is flown through the channel and pressure drops across the resistance structure of the Fleisch type mouthpiece and the corresponding flow rates are recorded. Using this data, exhaled flow rate is expressed as a 5th order polynomial transfer function of pressure. The transfer function is included in the Digital Signal Controller (DSC) to obtained volumetric flow rates based on the differential pressure. Accuracy of the flow sensor is tested using a 3L calibration syringe. 3L of ambient air is emptied into the mouthpiece at various flow rates. Flow rates obtained by the flow sensor are integrated with respect to time to derive volume passed through the sensor and compared with the actual volume passed through the sensor (3L). The flow meter meets the error standard (3% or less) required for clinical spirometers.

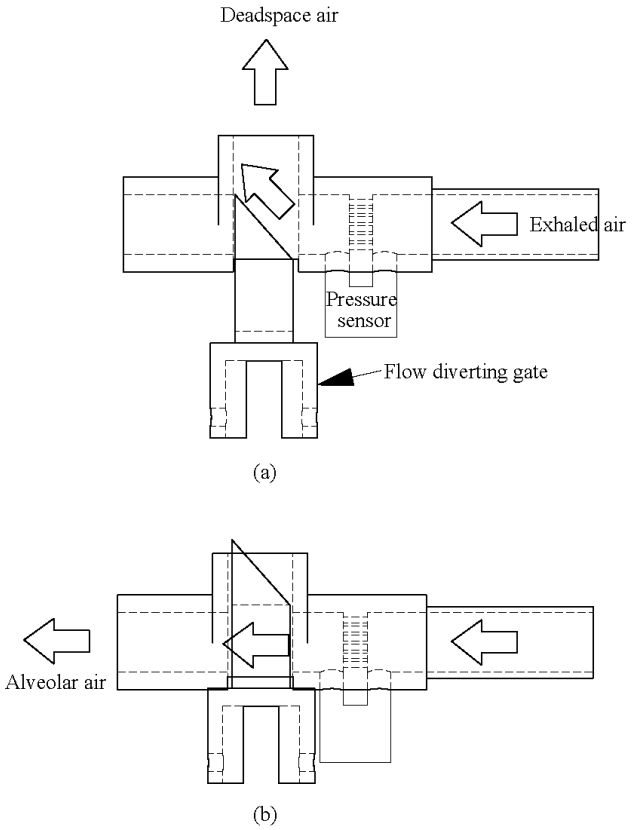

Figure 1. Alveolar flow separating device (a) Alveolar flow channel is closed and the device is diverting dead space air away from the alveolar air channel. (b) Alveolar flow channel is open and the device is directing alveolar air into the alveolar air channel.

B. Separating alveolar air from dead space air

For a young adult male, dead space volume is about 30 percent of the entire exhaled volume [1]. Dead space air is followed by alveolar air. Alveolar air separator calculates the average dead space volume of the first three breath cycles. This value is used as the threshold to determine the transition from dead space air to alveolar air. In the consecutive breath cycles, when the exhaled volume exceeds the threshold volume alveolar channel is opened. Alveolar channel remains closed during dead space air expiration and inhalation.

III. ALVEOLAR VOC EXTRACTING DEVICE

A schematic diagram of the device is shown in Fig. 2. The device directly extracts VOCs from exhaled air into a modified SPME filament, thus eliminate the need for collecting the entire breath volume over a multiple breath cycles into a large gas-sampling container. The criteria needed to use SPME filaments for direct breath sampling is the following. (1) Amount of VOCs extracted during the short alveolar portion of the breath is proportional to the concentration of the VOCs in the sample. (2) Extraction is independent from the flow rate. (3) Total exposure time is a constant.

Alveolar VOC extractor consists a modified SPME filament and a solenoid linear actuator. The linear actuator moves the filament out of the needle only when the alveolar channel is opened. The time SPME filament is exposed to alveolar air is continuously monitored. The third criteria is satisfied by maintaining the total exposure time constant using a timer.

First two criteria are experimentally validated and

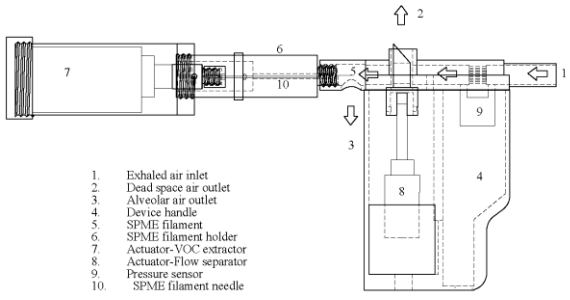

Figure 2. Alveolar breath VOC extracting device

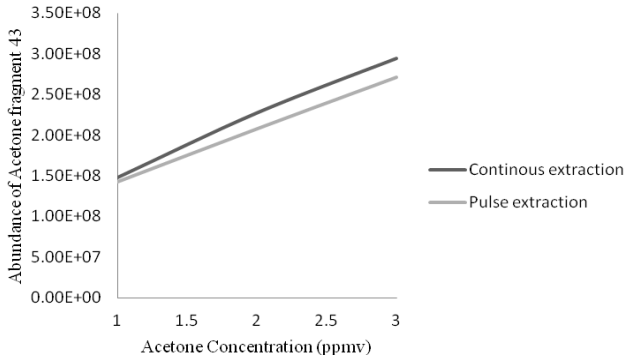

Figure 3. Abundance of acetone fragment 43 at various concentrations using continuous extraction method and pulse extraction method

described in the following sections.

A. Experimental verification of relationship between VOCs extracted into the filament and sample concentration at extremely short exposure time

1ppmv, 2ppmv and 3ppmv acetone gas standards are prepared in gas sampling bags. SPME filament is used to extract acetone from the gas sampling bag. The filament is exposed to the sample for 10 seconds continuously and this method of extraction is referred as continuous extraction. Abundance of acetone collected using the continuous extraction method is measured using GC/MS system and compared to pulse extraction method. In pulse extraction, the filament is exposed approximately one second at a time for a total exposure time of 10 seconds. The pulse extraction method is similar to SPME filament exposure pattern expected in the alveolar air VOC extractor. Experiments were conducted for all three different concentrations. Prior to each test SPME filament is kept in the GC for 5 minutes at 250°C and gas sampling bags are purged three times with Nitrogen gas. SPME filaments are extremely time sensitive at short exposure times. Following method is used to accurately measure the filament exposure time during the experiments. SPME syringe is mounted on a breadboard and the plunger of the syringe is physically connected to a linear potentiometer. Potentiometer is connected to ADC of a DSC. Corresponding voltages when filament is inside the needle and when the filament is outside the needle is determined and a threshold voltage for filament exposure is set. When threshold voltage is exceeded when the plunger is pressed, a timer is activated and the filament exposure time is recorded. Abundance of acetone fragment 43 at various concentration for continuous and pulse extraction are plotted in Fig. 3. Continuous and pulse extraction methods show similar abundance of acetone fragment 43. We believe slightly lower abundance in pulse extraction may due to over estimation the exposure time. The significant outcome of the evaluation is that the abundance of acetone collected using the pulse extraction method is linearly proportional to acetone concentration in the sample.

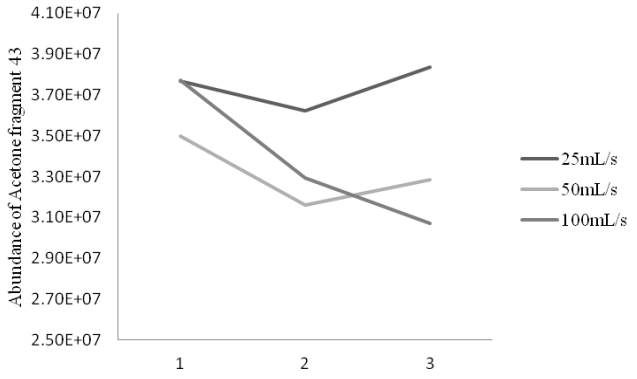

Figure 5. Abundance of Acetone fragment 43 at three different flow rates. Experiment of was repeated three times for each flow rate.

B. Experimental verification of relationship between VOCs extracted into the filament and sample concentration at different flow rates

Acetone gas standards are prepared in gas sampling bags. Gas sampling bags are connected to a vacuum pump. The pump regulates the flow rate by varying the supplied power. The outlet of the vacuum pump is connected to a tube where SPME filament can be inserted into the channel through a septum. SPME filament is exposed to the flow stream of acetone gas pumped out from the gas-sampling bag for constant amount of time. Experiment was repeated at different flow rates for different concentrations. Experiment is also conducted for dynamic flow rate change that mimics the exhaled flow rate. Based on the results showed in Fig. 5 there is no statistically significant difference among the abundance of extraction of acetone at different flow rates.

C. Device validation

Alveolar air VOC extractor is validated using the following method. Subject sits upright and breath through the device at a comfortable breathing rate. The device determines the threshold volume for dead space air at the end of the first three exhaled cycles and begins extracting VOCs from alveolar air starting from the fourth exhaled cycle. SPME filament is selectively exposed to alveolar air for total alveolar air exposure time of 1 minute. Once the VOC extraction is completed SPME filament is removed from the device and abundance of acetone is measured using the GC/MS system. Once sampling using the device is completed, alveolar air is manually collected into a gas-sampling bag. Subject exhales the last portion of the breath into the gas-sampling bag until the bag is filled with alveolar air. SPME filament is inserted in the gas-sampling bag for one minute and abundance of the acetone is measured. Acetone gas standards of 0.166ppmv, 0.333ppmv, 0.666ppmv, 1ppmv and 2ppmv are prepared for calibration purposes. SPME filament is exposed in each gas standard for one minute. Abundance of acetone extracted is measured and calibration curve is obtained. The calibration curved is then used to quantify acetone in breath samples collected using the device and gas sampling bag. Acetone concentration in exhaled breath determined by the sample obtained by the alveolar air VOC extractor is comparable to the acetone concentration in gas sampling bag and acetone concentration is within the range of a healthy person.

IV. Conclusion

The alveolar air VOC extractor is developed as a POC device for convenient breath sampling. A quantifiable breath sample is collected by simply breathing through the device for a few minutes. Although only acetone is quantified in device validation, the device's extraction is non-selective and abundance of other VOCs of interest can be measured using the same method. The sample container is compatible with most GC/MS systems. The device's ability to selectively extract VOCs from alveolar air is demonstrated. It also has the capability of selectively extracting VOCs from dead space air. This information is helpful in determining the origin of a VOC. In addition to clinical breath sampling, the device has applications in monitoring environmental VOC exposure.

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