Lasers in Medical Science (2019) 34:773–778
https://doi.org/10.1007/s10103-018-2661-z

ORIGINAL ARTICLE

Spectroscopic study of breath ethylene via the mouth and nose

A. M. Bratu[1]

Received: 13 March 2018 / Accepted: 7 October 2018 / Published online: 11 October 2018

Abstract

The development of new techniques for breath analysis searching for objective biomarkers of oxidative stress showed promise in non-invasive disclosing health information of the well-being of a person. Although numerous biomarkers have been identified so far using breath analysis, very little is known about their origin if they are metabolic or providing from mouth contamination. For the introduction of breath tests into clinical practice, standardization of sample collection needs to be taken into account. Breath analysis has been performed using laser photoacoustic spectroscopy to evaluate exhaled breath by mouth and nose before and after brushing with toothpaste/baking soda in order to identify the important endogenous biomarkers without contaminant sources. As a known biomarker of oxidative stress in the human body, it is important to accurately assess ethylene from exhaled air. Differences in the concentrations of exhaled ethylene are observed after using toothpaste and baking soda. The levels of ethylene are lower for nose breathing compared with mouth breathing. However, the differences are not significant proving that ethylene is generally endogenous but may still exist some contamination, depending of the oral hygiene of each person. These results may lead to a procedure, whereby subjects should be instructed to use toothpaste before each breath test sampling, to avoid the possibility of contamination of endogenous biomarkers.

Keywords Ethylene · Biomarker · Breath analysis · Mouth/nose · Laser photoacoustic spectroscopy

Introduction

Trace constituents of the exhaled breath of healthy humans generally have both endogenous and exogenous sources [1, 2]. The exogenous compounds are derived from environmental noxes, bacteria, and viruses, whereas endogenous compounds arise from metabolic processes within the body. They are released into the blood and will be passed on the airway via gas-exchange between the blood and alveolar air in the lungs [3–6] and appear in exhaled breath, being measured at trace concentrations in the parts-per-million by volume (ppmv) and parts-per-billion by volume (ppbv) levels or lower.

Breath biomarkers may be affected by bacterial growth in the oral cavity or in the upper respiratory tract [7, 8]. These bacteria are generally kept under control with a healthy diet and proper oral hygiene, brushing teeth at least twice a day. The most used products for oral hygiene are cosmetic toothpaste, baking soda, and alcohol mouthwashes. To better recognize an unhealthy breath, it is necessary to establish a healthy one. Although the health status of patients can be identified with a single breath, collecting a pure breath sample is not as simple as it may seem. The aspects of sampling procedure must be studied in detail in the case of measuring breath biomarkers.

The level of compounds in human breath can change slightly depending on every person oral hygiene (if these persons used care products before breath collection or not), and also on the time and day that the breath sample was taken. Therefore, each person has a unique "breath print" that remains distinct and relatively stable [9, 10].

Ethylene is a known biomarker for several metabolic disorders. Nevertheless, there are many concerns if this biomarker is truly endogenous or may have some exogenous sources. Exogenous sources of ethylene include the natural product from the vegetation of all type, burning of vegetation, degradation of agricultural wastes and refuse, active and passive smoking, and incomplete combustion of fossil fuels [11]. Endogenous sources of ethylene are formed from several possible sources, including lipid peroxidation of unsaturated fats, oxidation of free methionine, oxidation of hemin in

✉ A. M. Bratu
ana.magureanu@inflpr.ro

[1] Laser Department, National Institute for Laser, Plasma and Radiation Physics, 409 Atomistilor Str, P.O. Box MG-6, RO-077125 Bucharest, Magurele, Romania

hemoglobin, and metabolism by intestinal bacteria [12–14]. Ethylene resulting from lipid peroxidation known as a biomarker of oxidative stress in the human body was one of the first breath compounds studied, being reported to range between 3 and 100 ppb [15–18].

To investigate the feasibility of continuous assessment of lipid peroxidation by expired ethylene in the clinical applications, this study shows methods and procedures to provide concentrations of truly endogenous ethylene. These experiments were initiated to measure the ethylene in the breath exhaled via the *mouth* and *nose* and also to decide the influence of toothpaste and baking soda in the oral cavity.

These studies are an extension of previous breath analysis studies carried out using CO_2 laser photoacoustic spectroscopy. This technique presents flexibility, high stability, large dynamic range to measure low concentrations, good time response, and high sensitivity in detection. In addition, analyses of exhaled breath can be performed in real time which gives a clear advantage over blood tests [19].

Detection of ethylene concentration in breath with an acceptable accuracy can offer an indicator of oxidative stress in the human body, allowing a distinction between healthy and diseased states. Thus, breath tests are important because of their potential to detect diseases in early stages, noninvasively with no pain and discomfort.

Method

To distinguish endogenous biomarkers from contaminants originating from the environment or bacteria, *mouth*- and *nose*-exhaled breath have been investigated using the CO_2 laser photoacoustic spectroscopy. This method is very sensitive and selective and well known in the field of trace gas detection (ppb level). It was used in this study for quantitative determination in real time of ethylene, with operational simplicity, easy calibration, and no need for sample preparation.

CO_2 laser photoacoustic spectroscopy system has been described before in several research papers [1, 19–27]. The main components of the system are a frequency-stabilized line-tunable CO_2 laser and a resonant photoacoustic cell where the gas concentration is detected and analyzed in combination with a flow-through system. Multicomponent mixtures can be measured with high sensitivity and necessary selectivity by this system for the molecules that possess high absorption strengths and a characteristic absorption pattern in the wavelength range of the CO_2 laser [21–25] (Fig. 1).

The continuous wave CO_2 laser is frequency-stabilized emitting radiation in the 9.2–10.8-μm region with a maximum power of 6.5 W. The CO_2 laser beam is modulated in intensity by a high-quality low-vibration noise, and variable speed mechanical chopper operating at the appropriate resonant frequency of the cell (564 Hz) is focused by a ZnSe lens and then is introduced in the photoacoustic cell. The resonant photoacoustic cell was developed to monitor different gases. In the resonator tube wall, four microphones are carefully embedded where the acoustic wave is detected and generates a corresponding signal (voltage) which is fed into a lock-in amplifier. The lock-in amplifier gives the amplitude and the phase of the photoacoustic signal synchronized to the chopper phase. The amplitude of the photoacoustic signal is proportional to the concentration of the absorbing molecules. A powermeter measures the laser beam power after the photoacoustic cell. Its digital output is introduced in the data acquisition interface module together with the output from a lock-in amplifier. All experimental data are processed and stored by a computer. The modular software architecture for controlling the experiments, collecting data, and pre-processing information helps to automate the process of collecting and processing the experimental results.

The experiments were conducted using a gas handling and vacuum systems used to ensure gas purity in the photoacoustic cell, to pump out the cell, introduce the sample gas in the photoacoustic cell at a controlled flow rate, and monitor the pressures of gas mixtures. The gas handling system also includes two gas flow controllers, the sample bag and the potassium hydroxide scrubber. The breath samples were collected in special bags equipped with valves that sealed them after filling. The stored breath samples were transferred from bags into the measuring cell by the gas flow controller. Before entering the photoacoustic cell, the gas mixture passes through a potassium hydroxide scrubber, which retains most of the interfering carbon dioxide and water vapors [24].

The ethylene gas signature (see Fig. 2) was experimentally determined using commercially prepared, certified gas mixtures containing 0.996 ppmV ethylene diluted in pure nitrogen.

Also, the responsivity R (cm V W^{-1}) of the PA cell is an important parameter which depends on the pressure of the gas inside the PA cell. Taking into account the fact that the initial pressure in the sample bags filled by the human respiration differs from one case to another, it is necessary to know the pressure dependence of the PA cell responsivity (Fig. 3).

The pressure of the gas inside the cell influences the responsivity R (cm V W^{-1}) of the photoacoustic cell. The initial pressure in the sample bags filled by the healthy humans differs from subjects with different disorders and it is necessary to know the pressure dependence of the photoacoustic cell responsivity [24]. The exhaled air sample was transferred to the photoacoustic cell at a controlled flow rate of 36 L/h (600 sccm: standard cubic centimeters per minute), and the total pressure of the gas in the cell was measured, applying then the correction factor for the responsivity according to the calibration curve.

The volunteers collected the breath air sample in aluminized multipatient collection bags (750-mL aluminum-

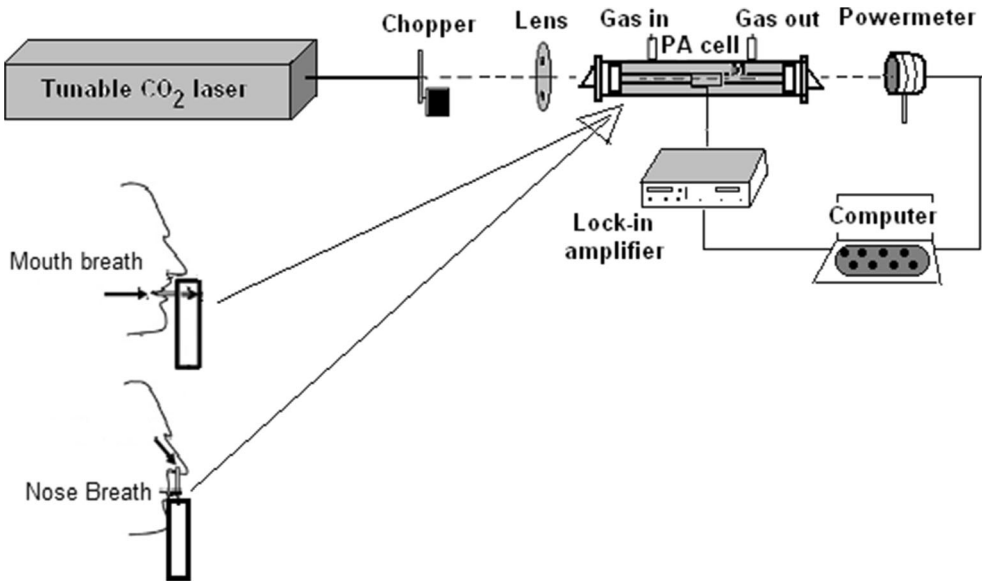

Fig. 1 Schematic of the CO_2 LPAS instrument

coated bags) composed of a disposable mouthpiece, a tee-mouthpiece assembly, a discard bag, and a tube for nasal breathing.

Multipatient collection bags are designed to collect multiple samples from patients and hold a sample for a maximum of 6 h. After an approximately normal inspiration, the subject places the mouthpiece in his mouth, forming a tight seal around it with the lips. A normal expiration is then made through the mouth, in order to empty the lungs of as much air as required to provide the breath sample. For the mouth-exhaled breath sample, the first portion of the expired air is directed into the discard bag (with the role in the collection of the "dead-space" air: the first portion of an expired breath), while the alveolar air is diverted to the collection bag. When an adequate sample is collected, the subject stops exhaling and removes the mouthpiece. For nasal breathing, a Teflon tube was used and was connected to the bag. The tube was inserted into one nostril while the other was locked with the finger. A normal expiration is then made through the nose, in order to empty the lungs of as much air as required to provide the breath sample. When an adequate sample is collected, the subject stops exhaling and removes the tube [24] (Fig. 4).

After the volunteer exhaled via the *mouth* or the *nose* and the sample is collected, the gas from the sample is transferred into the PA cell and can be analyzed immediately or later. In either case, it is recommendable to seal the large port with the collection bag port cap furnished with the collection bag. The use of the port cap assures that the sample volume will not be lost due to a leak. Its use also avoids the contamination of the sample by gas diffusion through the one-way valve in the large port, if the sample is stored for a long period of time prior to its analysis.

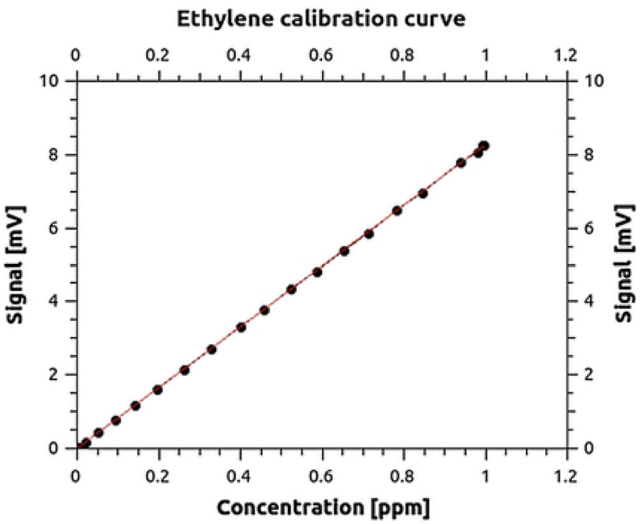

Fig. 2 The concentration-dependent response for ethylene

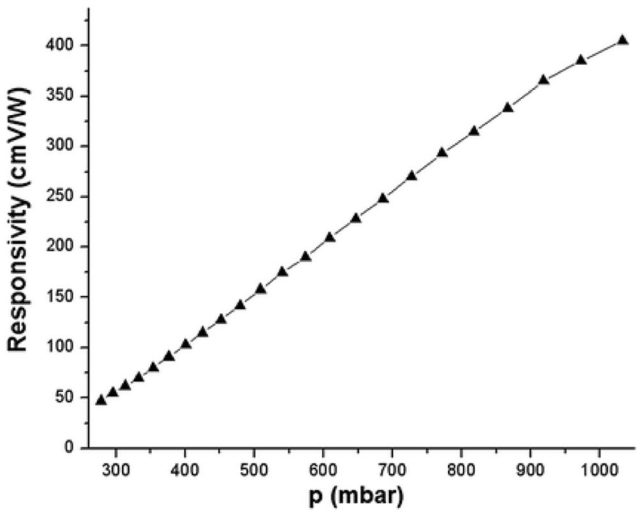

Fig. 3 The responsivity of the PA cell against the pressure

Fig. 4 The mouth- and nose-exhaled breath sample collection system

The volunteers were recruited from co-workers. All of subjects lived in the same region (Magurele) and had similar patterns of physical activity and similar levels of education. None of the assessed subjects had been exposed to ionizing radiation of antibiotics before study.

A detailed questionnaire with general information (age, gender) and anthropometric data (weight, height) as well as life style, health status, and dietary habits was filled in for each subject.

The subjects were informed about the aim and experimental details of the study and all give their written informed consent. No private details on the subjects involved in the study have been or will be disclosed in public.

In this study, 15 healthy (9 women and 6 men) volunteers with ages between 29 and 33 (co-workers of our institute) were involved. Healthy subjects were non- or ex-smokers, non-alcoholic, non-renal, non-diabetic without chronic mental or physical health problems, and without any recent antibiotic therapy. Prior to the analysis of breath, the volunteers were asked to avoid for at least 6 h before or at any time during the breath sample collection the following: alcohol and coffee, food or beverages, and to refrain from exercise in the morning. On the day prior to the test, products such as onions, leeks, eggs, and garlic were avoided. Information was asked regarding age, body weight and body height, time and nature of the last meal and drink, recent exercise activity, medication, and smoking. It should be pointed out that all the data published about the volunteers were undertaken with the understanding and written consent of each volunteer. All breath samples were given in the laboratory every day between 08:00 am and 13:00 pm over a period of 1 month.

Results and discussions

First, *mouth*-exhaled samples and *nose*-exhaled samples from volunteers were analyzed in the morning before washing teeth, eating, or drinking anything (baseline values). Then, a toothpaste study was conducted, where breath samples were taken before brushing and 10 min after brushing with toothpaste and rinsing with tap water.

The same procedure was used for baking soda. The exhaled air sample was transferred to the photoacoustic cell at a controlled flow rate of 600 sccm, and the total pressure of the gas in the cell was measured. To analyze the *mouth*- and *nose*-exhaled bags contents, the extra gas was evacuated firstly by the vacuum handling system, and the system was flushed with pure nitrogen at atmospheric pressure for 30 min. For determining the detection of ethylene gas, the CO_2 laser was kept

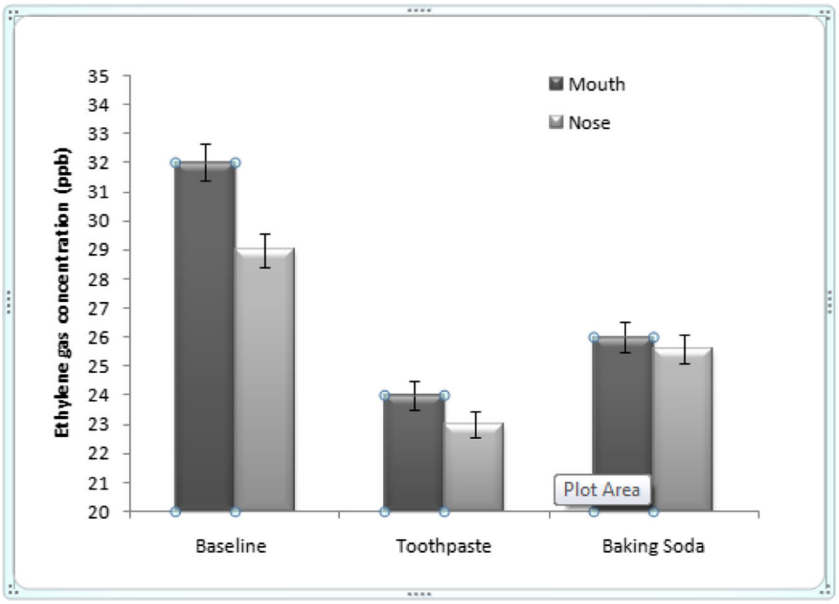

Fig. 5 The average concentration of ethylene exhaled by mouth and nose (with error bars)

tuned to the 10P (14) laser line where the gas exhibits the strong characteristic peak.

These comparative measurements were performed to analyze factors that potentially may affect the levels of ethylene in the breath and to standardize a method for measurement controlling breathing parameters.

Figure 5 shows the average concentrations of ethylene, both the *mouth*-exhaled breath and the *nose*-exhaled breath, before and after brushing with toothpaste and baking soda, followed by rinsing with tap water.

As can be seen in Fig. 3, baseline values of ethylene concentration are 32 ppb in the *mouth*-exhaled breath and 29 ppb for *nose*-exhaled breath. The most likely explanation for this is that a minor contributor to the ethylene in *mouth*-exhaled breath is oral bacteria. There is no significant difference between the *mouth* and *nose* breath levels for ethylene when baking soda is used but still, levels of ethylene are lower than baseline values: 26 ppb *mouth* and 25.5 ppb *nose*. The lowest levels were found after using toothpaste. For *mouth*-exhaled breath, ethylene level was 24 ppb and for *nose*-exhaled breath was 23 ppb.

The *nose* levels were always lower than the *mouth* levels but the difference was not significant indicating that this compound is mostly endogenous being released at the alveolar interface and is not produced in measurable amounts in the oral cavity.

To diagnose metabolic diseases, the volunteers should be instructed to use toothpaste before each breath test sampling to avoid oral bacteria [30] (over 700 species of bacteria live in our mouth and can interfere with our molecules of interest).

Conclusions

Results have been performed using real-time CO_2 laser photoacoustic spectroscopy for the breath of 15 healthy volunteers, as they exhaled via the *mouth* and *nose* after brushing with toothpaste and baking soda, each morning over a period of 1 month. Ethylene trace compounds have been quantified and concentration distributions have been obtained.

The main conclusion of the study is that ethylene is endogenous and appears in the exhaled breath of all volunteers. The data obtained clearly show that ethylene exhaled via the *mouth* does not provide only alveolar but still, the mouth production is minimal. According to this study, it is essential to develop and perform a routine, standard procedure for breath samples. The procedure of brushing with toothpaste must be followed before taking samples.

Generally, the level of compounds in human breath can change slightly depending on every person oral hygiene, on the time and day that the breath sample was taken, each person having a unique chemical composition that remain distinct and relatively stable. More extensive studies with a larger number of volunteers using mouth and nose monitoring before and after brushing with toothpaste may add clarity to this preliminary research by keeping the present determinations as a reference.

Exhaled breath sample can allow rapid progress in the area of clinical applications because the procedure is non-invasive and breath can be sampled as often as necessary, continuously even during sleep [28, 29] as opposed to blood, which cannot be sampled continuously.

CO_2 laser photoacoustic spectroscopy has the potential to be a viable tool for monitoring real-time concentrations of ethylene in human respiration. Being able to extract a signal from flowing breath samples, it provided the advantage of collecting large amounts of data in a short period of time.

Currently, there are no tools available with these features in the clinical procedures, so controls are based on complex laboratory analyses that require long times and specialized personnel. The results of this study are hoping to arrive shortly for quick, reliable, and simple execution controls.

The CO_2 laser photoacoustic spectroscopy is admirably suited for the measurement of ethylene in exhaled breath, and the reported breath measurements made using this technique are perhaps the most reliable measurements available.

Funding information This work was supported by a grant of the Ministry of National Education and Scientific Research, RDI Program for Space Technology and Advanced Research—STAR, project number 153.

Compliance with ethical standards

Informed consent For this research, I have the consent of the participants. The time and effort provided by the volunteers is greatly appreciated.

Conflict of interest The author declares that she has no conflict of interest.

References

1. Pleil JD, Stiegel MA, Risby TH (2013) Clinical breath analysis: discriminating between human endogenous compounds and exogenous (environmental) chemical confounders. J Breath Res 7(1): 017107. https://doi.org/10.1088/17527155/7/1/017107
2. Phillips M, Herrera J, Krishnan S, Zain M, Greenberg J, Cataneo RN (1999) Variation in volatile organic compounds in the breath of normal humans. J Chromatogr B Biomed Sci Appl 729:75–88
3. Schnabel R, Fijten R, Smolinska A, Dallinga J, Boumans M-L, Stobberingh E, Boots A, Roekaerts P, Bergmans D, van Schooten FJ (2015) Analysis of volatile organic compounds in exhaled breath to diagnose ventilator-associated pneumonia. Sci Rep 5: Article number 17179. https://doi.org/10.1038/srep17179
4. Manolis A (1983) The diagnostic potential of breath analysis. Clin Chem 29:5–15
5. Smit D, Turner C, Spanel P (2007) Volatile metabolites in the exhaled breath of healthy volunteers: their levels and distributions. J Breath Res 1:014004 (12pp)

6. Shirasu M, Touhara K (2011) The scent of desease:volatile organic compounds of the human body related to disease and disorder. J Biochem 150(3):257–266
7. Palmer Jr RJ (2011) Composition and development of oral bacterial communities. Periodontol 2000 64(1). https://doi.org/10.1111/j.1600-0757.2012.00453.x
8. Gomes-Filho IS, Passos JS, Seixas da Cruz S (2010) Respiratory disease and the role of oral bacteria. J Oral Microbiol 2. https://doi.org/10.3402/jom.v2i0.5811
9. Haick H, Broza YY, Mochalski P, Ruzsanyi V, Amann A (2014) Assessment, origin, and implementation of breath volatile cancer markers. Chem Soc Rev 43(5):1423–1429
10. Gouma P, Stanacevic M (2011) Selective nanosensor array microsystem for exhaled breath analysis. Procedia Eng 25:1557–1560. https://doi.org/10.1016/j.proeng.2011.12.385
11. IARC ethylene (1994) IARC scientific publications no. 60. IARC, Lyon, pp 45–71
12. Törnqvist M, Kautiainen A, Gatz RN, Ehrenberg L (1988) Hemoglobin adducts in animals exposed to gasoline and diesel exhausts 1. Alkenes J Appl Toxicol 8:159–170
13. Törnqvist M, Gustafsson B, Kautiainen A, Harms-Ringdahl M, Granath F, Ehrenberg L (1989) Unsaturated lipids and intestinal bacteria as sources of endogenous production of ethene and ethylene oxide. Carcinogenesis 10:39–41
14. Törnqvist M (1989) Search for unknown adducts: increase of sensitivity through preselection by biochemical parameters. In: Bartsch H, Hemminki K, O'Neill IK (eds) Methods for detecting DNA damaging agents in humans: applications in cancer epidemiology and prevention. IARC Scientific Publications no. 89. IARC, Lyon, pp 378–383
15. Popa C, Bratu AM, Cernat R, Dutu DCA, Banita S, Dumitras DC (2011) Spectroscopic studies of ethylene and ammonia as biomarkers at patients with different medical disorders. U P B Sci Bull Ser A 73:167–174
16. Petrus M, Bratu AM, Popa C (2017) Spectroscopic analysis of breath ethylene and oxidative stress relation with glycaemic status in type 2 diabetes. Opt Quant Electron 49(2). https://doi.org/10.1007/s11082-016-0837-y
17. Popa C, Bratu AM, Matei C, Cernat R, Popescu A, Dumitras DC (2011) Qualitative and quantitative determination of human biomarkers by laser photoacoustic spectroscopy methods. Laser Phys 21:1336–1342
18. Dumitras DC, Dutu DC, Matei C, Magureanu AM, Petrus M, Popa C, Patachia M (2008) Measurements of ethylene concentrations by laser photoacoustic techniques with applications at breath analyses. Rom Rep Phys 60:593–602
19. Dumitras DC, Dutu DC, Matei C, Magureanu AM, Petrus M, Popa C (2007) Laser photoacoustic spectroscopy: principals, instrumentation and characterization. J Optoelectron Adv Mater 9:3655–3701
20. Dumitras DC, Banita S, Bratu AM, Cernat R, Dutu DCA, Matei C, Patachia M, Petrus M, Popa C (2010) Ultrasensitive CO_2 laser photoacoustic system. J Infrared Phys Technol 53:308–314
21. Petrus M, Bratu AM, Popa C (2016) The response of human body at oxidative stress in subjects with type 2 diabets: ammonia breath analysis by laser photoacoustic spectroscopy. Rev Roum Chim 61(2):89–95
22. Popa C (2016) Breathing disorders using photoacoustics gas analyzer. J Med Imaging Health Inform 6:1893–1895
23. Cernat R, Matei C, Bratu AM, Dutu DC, Patachia M, Petrus M, Banita S, Dumitras DC (2010) Laser photoacoustic spectroscopy method for measurements of trace gas concentration from human breath. Rom Rep Phys 62:610–616
24. Bratu AM, Popa C, Matei C, Banita S, Dutu DCA, Dumitras DC (2011) Removal of interfering gases in breath biomarker measurements. J Optoelectron Adv Mater 13:1045–1050
25. Popa C, Patachia M, Banita S, Dumitras DC (2013) Exercition in Kangoo Jumps aerobic: evaluation and interpretation using spectroscopic techniques determinations. J Spectrosc Article ID 602434. https://doi.org/10.1155/2013/602434
26. Liu W, Wang L, Li L, Liu J, Liu FQ, Wang Z (2011) Fast simultaneous measurement of multi-gases using quantum cascade laser photoacoustic spectroscopy. Appl Phys B 103(3):743
27. Li J, Gao X, Fang L, Zhang W, Cha H (2007) Resonant photoacoustic detection of trace gas with DFB diode laser. Opt Laser Technol 39(6):1144
28. King J, Mochalski P, Kupferthaler A, Unterkofler K, Filipiak W, Teschl S, Teschl G, Hinterhuber H, Amann A (2010) Dynamic profiles of volatile organic compounds in exhaled breath as determined by a coupled PTR-MS/GC-MS study. Physiol Meas 31:1169–1184
29. King J, Mochalski P, Unterkofler K, Teschl G, Klieber M, Stein M, Amann A, Baumann M (2012) Breath isoprene: muscle dystrophy patients support the concept of a pool of isoprenein the periphery of the human body. Biochem Biophys Res Commun 423:526–530
30. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade WG, Zaura E (2016) The oral microbiome – an update for oral healthcare professionals. Br Dent J 221:657–666