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Determination of Volatile Organic Compounds in Human Breath by Optical Fiber Sensing

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***Abstract*—**This work proposes an optical fiber system (OF) for sensing various volatile organic compounds (VOCs) in human breath for the diagnosis of some metabolic disorders as a non-invasive methodology. The analyzed VOCs are alkanes (i.e., ethane, pentane, heptane, octane, and decane), and aromatic compounds (i.e., benzene, toluene, and styrene). The OF displays high analytical performance since it provides near real-time responses, rapid analysis, and low instrumentation costs, as well as it exhibits useful linear range and detection limits; the developed OF sensor is also comparable to a reference methodology (gas chromatography-mass spectrometry) for the eight tested VOCs.

***Keywords*—**Breath analysis, gas chromatography-mass spectrometry, optical fiber sensor, volatile organic compounds

I. Introduction

THE monitoring of the changes in the composition of human breath is crucial for the diagnosis of some metabolic disorders (e.g., diabetes, potassium and magnesium metabolism disorders), lung diseases (e.g., asthma, cystic fibrosis, and bronchiectasis), oxidative stress, and gastrointestinal disease [1]. The breath testing constitutes a non-invasive diagnostic method which can also be used for the monitoring of therapeutic intervention, drug monitoring, and the progression of diseases.

The exhaled breath is composed by a mixture of inorganic gases (e.g., carbon monoxide, carbon dioxide, and nitric oxide), water, inert gases, and volatile organic compounds (VOCs) such as saturated (e.g., ethane and pentane) and unsaturated (e.g., isoprene) hydrocarbons, oxygen-containing (e.g., acetone), sulfur-containing (e.g., dimethylsulfide), and nitrogen-containing compounds (e.g., ammonia), which are present in concentrations from sub parts per billion (ppb) to parts per trillion (ppt) by volume [2], [3].

Specifically, the detection and quantification of some VOCs, i.e., alkanes (e.g., heptane, octane and decane) and aromatic compounds (e.g., toluene, benzene, and styrene) can allow to the diagnosis of diseases such as diabetes, airway inflammation, hepatic dysfunctions, and lung diseases [1], [3].

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Such compounds also function as breath biomarkers which are useful and specific for certain metabolic disorders or diseases. For example, the formaldehyde is associated to breast cancer, the isoprene to blood cholesterol levels, acetone to type 1 diabetes and lung cancer, ammonia to renal diseases, and pentane is associated to peroxidation of lipids and rheumatoid arthritis [3]. The breath biomarkers can be endogenous if they are produced in the body through cellular biochemical processes, or they can be exogenous if they are inhaled and absorbed though the lungs or absorbed through the skin [2]. As the exhaled breath has low concentrations of analytes and large quantity of trace compounds, sensitive and selective analytical instrumentations have been required to the accurate determination of VOCs and other specific biomarkers in exhaled air. The existing analytical techniques commonly used in laboratory such as proton-transfer reaction mass spectrometry, gas chromatography coupled to mass spectrometry (GC-MS), to infrared spectrometry (GC-IR), or to flame ionization detection (GC-FID), direct-injection mass spectrometry, and laser-absorption spectroscopy are sensitive and selective but they require expensive instrumentation and complex procedures for sampling and analysis preparation, as well as they are commonly used for laboratory research and not real-time techniques [1], [3]-[5]. Sensors arrays [6], [7] are also been recently proposed for analysis of acetone and alcohols, respectively; such systems are based on miniaturized instrumentation but they require frequent calibrations.The use of optical-chemical sensors constitutes another alternative with high analytical interest and advantages as unmatched sensitivity, non-invasiveness, and multiple detection of analytes from chemical, biological and clinical areas [8]-[12]. Based on such considerations, this work proposes a sensing system based on optical fiber (OF) transducer for clinical diagnosis as a competitive technique compared to the existing methodologies for the real-time determination of various VOCs, i.e., alkanes (ethane, pentane, heptane, octane, and decane), and aromatic compounds (benzene, toluene, and styrene) from human breath samples.

II. Materials and Methods

A. Sampling and Collection of Exhaled Breath, and Subjects Profiling

A group of 10 males and 10 females was subjected to a questionnaire where such volunteers have indicated their professional activity and personal habits, among other topics.All subjects were aged between 20 and 30 years old, and they were considered as healthy persons being non-smokers. The exhaled breath of each of the 20 subjects was collected for the measurement of eight selected VOCs, i.e., alkanes (ethane, pentane, heptane, octane, and decane), and aromatic compounds (benzene, toluene, and styrene), after a period of three hours where the subjects have not eaten or been submitted to physical activity.

The collection of breath samples was done on a laboratory under controlled conditions in terms of ventilation and temperature; the exhalation was done through a disposable mouthpiece (28 mm x 60 mm, medical express, Portugal) which was connected to a long tube (50 cm) through a Teflon plug, which in turn was connected to the sampling bulb (150 mL). For each subject tested, five breath samples, and one room air sample were collected.

B. Chemicals and Preparation of Standard Mixtures

The preparation of standards (purity > 98%, from Sigma-Aldrich and Fluka) was made with carbon disulfide. Five standards (25.0 mL) were prepared through serial dilutions of stock solutions (pentane, heptane, octane, decane, benzene, toluene, and styrene previously prepared in 50.0 mL volumetric flasks). The solutions were mixed in an ultrasonic bath for 5 min., and refrigerated at 4 ºC. The ethane gas standard was diluted in N_2 (99.999%, Praxair, Portugal). Table I provides the different concentrations of the VOCs standards used in this study, which were injected (5 µL) into the glass sampling bulb (SB) with a micro-syringe.

TABLE I
CONCENTRATIONS OF VOCS STANDARDS

VOC	Concentration (pmol.L^{-1})
Ethane	100, 200, 300, 400, and 500
Pentane	50, 150, 250, 350, and 450
Heptane	5, 10, 15, 20, and 25
Octane	2, 12, 22, 42, and 62
Decane	5, 125, 225, 325, and 425
Benzene	10, 40, 70, 100, and 130
Toluene	30, 60, 90, 120, and 150
Styrene	2, 22, 42, 62, and 82

C. Optical Fiber Sensing: Experimental Set-Up

Figure 1 shows the experimental set-up for the analytical system based on OF detection.

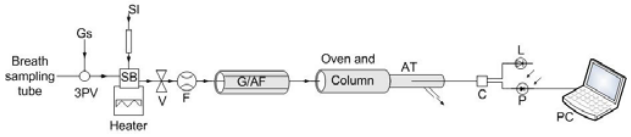

Fig. 1 Experimental configuration of the developed OF sensor for VOCs in standard and breath samples (Gs - carrier gas; 3PV - 3-port valve; SB - sampling bulb; SI - standards injection; V - valve; F - flow meter; G/AF - glass column/adsorbent fibers; AT - analytical tube; C - optical coupler; L - laser; P - photodetector; PC - personal computer)

After the vaporization of the samples (remained 20 min. in a glass column containing two adsorbent fibers, G/AF, with a constant flow of N_2, 25 mL.min^{-1}), the analytes were desorbed at 250 ºC. Then, the analytes were separated in a miniaturized column (70 mm x 4 mm o.d.) with polydimethylsiloxane (PDMS) as the coating matrix, under controlled conditions (the initial temperature was about 50 ºC, and then the temperature was increased until 100 ºC at 10 ºC.min^{-1} and to 250 ºC at 30 ºC.min^{-1}). The analytes was then detected by OF sensing after the carrying of the analytes through a constant flow of N_2 to the analytical tube (AT).

A 15 mm of pigtail (9 and 125 µm of diameter, for the core and cladding, respectively) covered by a 2 nm-thick of a siloxane polymer was inserted into the analytical tube after suitable preparation (mechanical uncladding, cleaning with dichloromethane, cleaving with a precision fiber cleaver, and cure of the OF end). The spray technique was used to deposit a coating film of poly[methyl(3,3,3-trifluoropropyl)siloxane] (0.05% in dichloromethane) on the cleaved OF end, which was cured at 70 ºC for 12 h. For the reliable breath analysis, and reducing potential interferences, the materials for the connection of the different components of the sensor and sampling tubes was made of Teflon, and the analytical tube (AT), columns and sampling bulb were made of glass, due to the adequate optical and physical properties of such materials.

Before the collection, the sampling bulb (SB) was purged with N_2 (99.999%, Praxair, Portugal) to reduce the ambient air contamination free from chemical and microorganisms.

The analytical principle of detection was based on the registration of changes in optical power when the analytes were contacted with the sensitive region of the OF end. Due to the different refractive indexes of the analyte molecules and the sensitive polymeric film, the optical change was proportional to the concentration of each analyte. A 1550 nm laser diode (L, Oz Optics, Canada) was used as the source of light, and a photodetector (P, Oz Optics, Canada) was used to detect the changes of the optical power; the data acquisition was performed on a personal computer (PC) by a home-made software.

D. Reference Methodology (Gas Chromatography-Mass Spectrometry, GC-MS)

The measurement of the eight selected VOCs (on standards and breath samples) was also performed by a reference methodology based on gas chromatography-mass spectrometry with a Shimadzu GC-17A GC coupled to a Shimadzu QP5000 GC-MS instrument for the comparison of the analytical results. Prior the analysis, VOCs standards and breath samples were extracted by solid phase microextraction; for that, a 75 µm Carboxen/PDMS fiber (Supelco) was introduced on the sampling bulb for 20 min. at the laboratory temperature and then the extracts were thermally desorbed in the GC injection port at 250 ºC. Then, the VOCs were separated on an Equity-1 column (30 m and 0.25 mm i.d., Supelco) at 50 kPa which was kept at 60 ºC for 5 min., then heated to 200 ºC (at 5 ºC.min^{-1}), and finally maintained at 200 ºC for 20 min.

III. RESULTS AND DISCUSSION

Before the analysis of collected breath samples by OF sensor and GC-MS as reference methodology, the injection of various standards was performed according to the concentrations identified on the Table I. The resulting calibration curves of the eight tested VOCs were shown in Figures 2 and 3, where the linear equations ($y = a + bx$), the coefficients of correlation (R^2) and respective p values as well as the limit of detection (LOD) calculated as three times the residual standard deviation were identified for each alkane (Figure 2), and aromatic compound (Figure 3).

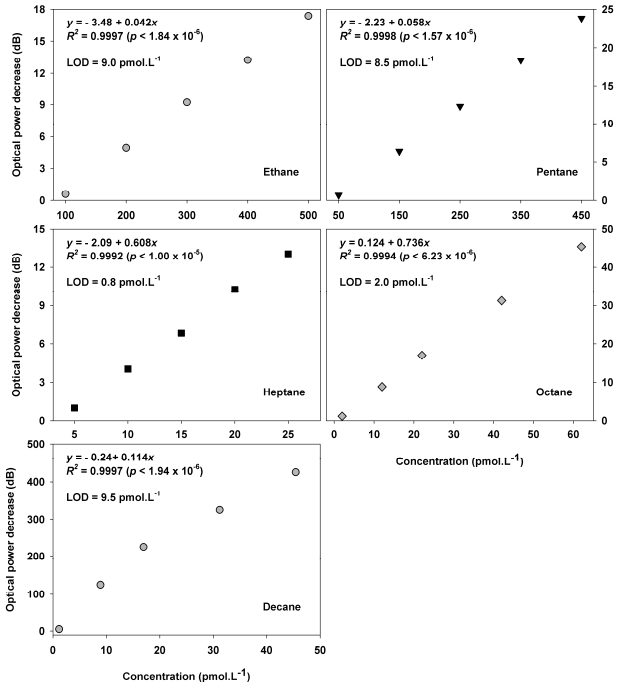

Fig. 2 Analytical responses obtained by OF sensor for the alkanes standards solutions, and corresponding analytical parameters

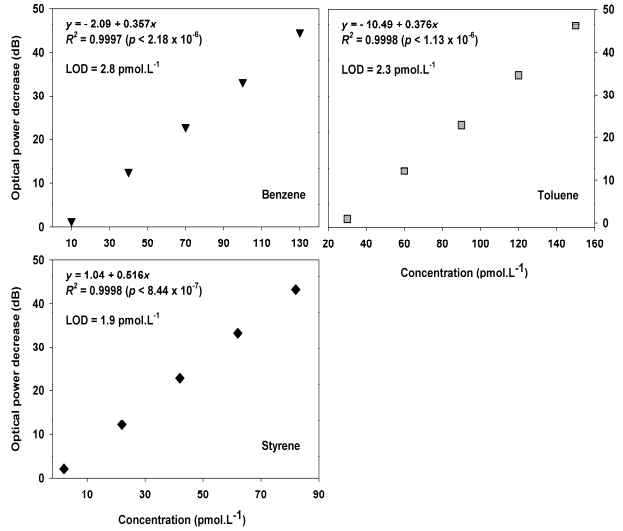

Fig. 3 Analytical responses obtained by OF sensor for the standards solutions of aromatic compounds, and corresponding analytical parameters.

From Figures 2 and 3, all VOCs demonstrated a high linearity due to the high values for the correlation coefficient (R^2). Low LODs were also found for the eight tested compounds in the order of pmol.L^{-1} and ranging from 0.8 pmol.L^{-1} to 9.5 pmol.L^{-1} for heptane and decane, respectively. The LODs from OF sensor were lower than that with GC-MS as shown in Table II, which is advantageous for the detection of VOCs on breath samples.

TABLE II
COMPARISON OF THE TWO METHODOLOGIES FOR THE DETECTION OF VOCS IN THE 20 BREATH SAMPLES

VOC	Mean concentration[1] and LOD (pmol.L^{-1})		Comparison between the two methodologies	
	OF	GC-MS	R^2	p value
Ethane	314.057 (9.0)	314.365 (14.3)	0.9997	3.11 x 10^{-33}
Pentane	275.358 (8.5)	274.665 (11.6)	0.9998	7.13 x 10^{-35}
Heptane	8.337 (0.8)	8.489 (0.8)	0.9925	1.40 x 10^{-20}
Octane	19.767 (2.0)	19.950 (2.0)	0.9988	8.26 x 10^{-28}
Decane	201.332 (9.5)	200.139 (14.1)	0.9999	5.91 x 10^{-36}
Benzene	42.832 (2.8)	43.709 (5.0)	0.9995	6.74 x 10^{-31}
Toluene	84.369 (2.3)	85.025 (3.4)	0.9991	1.05 x 10^{-28}
Styrene	14.025 (1.9)	14.425 (3.6)	0.9981	5.50 x 10^{-26}

[1]Mean concentration for the 20 breath samples

In Table II, the analytical parameters of the developed OF methodology (Figure 1) and the GC-MS as a reference method are present, and the two methodologies are found to be comparable. A linear correlation was obtained between the two methodologies for the eight tested analytes (with R^2 between 0.9925 and 0.9999, and $p < 1.40\text{x}10^{-20}$ and $p < 5.91\text{x}10^{-36}$, for heptane and decane, respectively), as verified in Table II. The OF system also provided narrow intervals at 95% confidence level, suggesting low dispersion levels of the results obtained by referred methodologies.

Furthermore, there is not a statistically significant difference between OF and GC-MS methodologies for the determination of the eight VOCs in breath samples, i.e., p values are about 0.903, 0.984, 0.914, 0.797, 0.977, 0.715, 0.797, and 0.756 for ethane, pentane, heptane, octane, decane, benzene, toluene, and styrene, respectively.

From Table II, it was also observed that the alkane with the higher average concentration on breath samples was the ethane, and the toluene was the most abundant aromatic compound on exhaled breath.

Furthermore, the levels of VOCs recorded in all breath samples collected for the healthy subjects are in accordance to the normal levels observed in other literature [13]; for example, the concentration levels commonly found in healthy subjects for heptane, octane, toluene, and styrene are in the order of 5.0-15.3 pmol.L^{-1}, 4.0-50.8 pmol.L^{-1}, 58.9-140.0 pmol.L^{-1}, and 5.3-21.8 pmol.L^{-1}, respectively, which cover the average concentration of the VOCs detected from breath samples in this work.

Interference assays in the breath analysis were also performed with 2-propanol (which is a disinfectant commonly used for the large skin areas of patients) and carbon disulfide (which is the solvent of all standards mixture prepared in this study) to evaluate the possible application of the developed OF sensor for clinical purposes; neither the 2-propanol nor the carbon disulfide were interfered with the analytical response of the OF sensor.

IV. CONCLUSION

Through the obtained analytical results, the developed OF sensor provides an adequate analytical performance based on the low LODs, linearity, and accuracy for the analysis of the tested eight VOCs in breath samples.

Such analytical performance was also equivalent with that of a reference methodology (GC-MS). In addition, the in-line, real-time, low-cost, simple and fast analysis of breath samples are the advantages of the developed OF sensor.

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