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Breath analysis by optical fiber sensor for the determination of exhaled organic compounds with a view to diagnostics

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ABSTRACT

Breath analysis constitutes a promising tool in clinical and analytical fields due to its high potential for non-invasive diagnostics of metabolic disorders and monitoring of disease status. An optical fiber (OF) sensor has been developed for determination of volatile organic compounds (ethane, pentane, heptane, octane, decane, benzene, toluene and styrene) in human breath for clinical diagnosis.

The analytical system developed showed a high performance for breath analysis, inferred for the analytical signal intensity and stability, linear range, and detection limits ranging from 0.8 pmol L^{-1}, for heptane, and to 9.5 pmol L^{-1}, for decane. The OF sensor also showed advantageous features of near real-time response and low instrumentation costs, besides showing an analytical performance equivalent to the breath analysis by gas chromatography–mass spectrometry (GC–MS), used as the reference method.

1. Introduction

The monitoring of exhaled breath is one of the most desirable techniques for non-invasive clinical diagnosis of diseases, disease progression, therapeutic intervention monitoring, and assessment of exposure to environmental pollutants [1–7]. Human breath matrix is a mixture of N_2, O_2, CO_2, H_2O, and a small fraction of a plethora of different volatile organic compounds (VOCs) with concentrations that can vary from sub parts per million (ppm) to parts per trillion (ppt) by volume [1].

The detection and quantification of certain VOCs, i.e., alkanes and aromatic hydrocarbons, in breath samples can provide clinically useful information for the diagnosis of a number of diseases, such as lung cancer, inflammatory lung diseases, hepatic dysfunction, and lipid peroxidation [3,4]. These compounds may be either generated in the body by biochemical reactions as a part of the metabolic process or withdrawn from the environment as contaminants [1,2]. From the VOCs profile found in human breath, many different "biomarker" molecules have been correlated to particular diseases and metabolic disorders [7–9]. The correlation between a biomarker and a specific disease is often multifold characterized; a chemical found in exhaled air can biomarker more than one disease or metabolic disorder, for example, ethane is a biomarker for vitamin E deficiency, lipid peroxidation, and asthma [7]. Due to the low concentrations and diversity of volatile compounds present in exhaled air, breath analysis requires a highly sensitive and selective analytical instrumentation in order to identify and accurately determine the volatile biomarker. Different techniques have been used for breath profiling, such as gas chromatography coupled to mass spectrometry (GC–MS) [10,11], proton transfer reaction mass spectrometry (PTR-MS) [12,13], vacuum-free ion mobility spectroscopy (IMS) with a multi-capillary column [14], selected ion flow tube mass spectrometry (SIFT-MS) [15–17], and GC coupled to flame ionization detection (GC–FID) [18,19]. These methods have routine detection limits, varying from ppb to ppt levels, allowing the selective analysis of multiple compounds in a single run. Although sensitive and selective, they require complicated procedures for sample collection and preparation for analysis [9–19], high analytical costs due to the expensive instrumentation needed, and even nowadays such equipment is reserved for laboratory research on breath analysis, beyond the challenge of implementation of an affordable and real-time analysis technique.

Although there has been an upward trend in the number of studies reporting breath analysis, a fast, simple and inexpensive analytical instrumentation to increase the potential use of exhaled organics with a view to diagnostics has yet to be developed. Laser absorption spectroscopic techniques seem to be as innovative methodologies for breath analysis [20,21]; 14 of the established breath biomarkers, i.e., ethane, ammonia, acetone, nitric oxide, carbon dioxide and carbon monoxide, have been analyzed by these techniques, as discussed by Wang and Sahay [7] in a study concerning high-sensitivity laser spectroscopic techniques for breath analysis. The main challenges of these methodologies are spectral interferences, effective application in clinical environment, and breath sampling. Breath analysis has also been conducted by using electrical sensors of small scale instrumenta-

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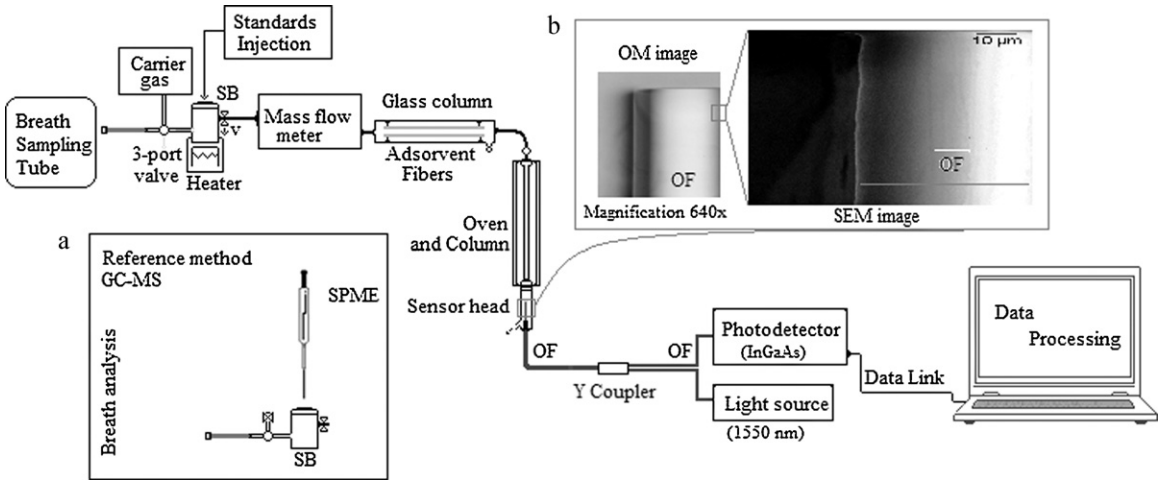

Fig. 1. Schematic configuration of the analytical apparatus used for determination of volatile organic compounds in exhaled air (OF – optical fiber, SB – sampling bulb, v – valve; SPME – solid phase microextraction). The inset figures show (a) the SPME sampling procedure used for the GC–MS breath analysis reference method and (b) the optical microscopy (MO) image and scanning electron microscopy (SEM) image of the sensor head.

tion; however, they have intrinsic low detection selectivity and require frequent calibrations [22–24]. Optical-chemical sensors have notability experienced high analytical interest and successful applications [25–34]; this type of device displays several analytical advantages over other sensing methods, such as unmatched sensitivity, remote measuring capabilities *via* optical fibers, non-invasiveness and multi-analyte detection of chemical, biological and clinical relevance [32,33]. The use of optical fiber in sensor systems seems to be a highly competitive technique for a wide range of application, including breath analysis, as highlighted by Wolfbeis et al. [35] in the development of an OF-based fluorescence sensor for narcotic halothane determination in the presence of oxygen; Mills et al. [36] for breath-by-breath carbon dioxide analysis using an OF sensor; DuBois et al. [37] in a study regarding the development of an OF sensor for ammonia detection in the breath of non-healthy subjects and the evaluation of the correlation of breath and arterial ammonia levels; and Mitsubayashi et al. [38] in an optical bio-sniffer for methyl mercaptan in halitosis.

This work aimed to develop an optical fiber (OF) sensor for real-time analysis of exhaled volatile organic compounds, such as ethane, pentane, heptane, octane, decane, benzene, toluene and styrene, which are breath biomarkers for a number of metabolic disorders, i.e., vitamin E deficiency, lipid peroxidation, lung cancer, oxidative stress, airway inflammation and rheumatoid arthritis. To meet this goal, the analytical potential of the OF device for fast, accurate, reliable and simple non-invasive breath monitoring, and its application for clinical diagnosis were assessed.

2. Experimental

2.1. Analysis design and subjects

The design of the reported study included the measurement of eight selected VOCs (ethane, pentane, heptane, octane, decane, benzene, toluene and styrene) in air exhaled by 20 healthy subjects (10 females and 10 males). Before breath collection, the healthy volunteer subjects had filled in a questionnaire regarding professional activity and personal habits, among other topics. All subjects were non-smokers, aged between 20 and 30 years old. For comparison purposes the breath analysis was performed not only by an OF-based analytical method but also by a GC–MS reference methodology.

2.2. Optical fiber sensor: analytical details and experimental set-up

The configuration of the analytical system developed based on OF detection is shown in Fig. 1, which also contains an inset figure showing the solid phase micro extraction (SPME) used for sampling the exhaled air for GC–MS analysis. For sensor system calibration, 5 μL of different concentrations of volatile standard mixtures were injected into the glass sampling bulb (SB), that is: (a) ethane: 100, 200, 300, 400 and 500 pmol L^{-1}; (b) pentane: 50, 150, 250, 350 and 450 pmol L^{-1}; (c) heptane: 5, 10, 15, 20 and 25 pmol L^{-1}; (d) octane: 2, 12, 22, 42 and 62 pmol L^{-1}; (e) decane: 5, 125, 225, 325 and 425 pmol L^{-1}; (f) benzene: 10, 40, 70, 100 and 130 pmol L^{-1}; (g) toluene: 30, 60, 90, 120 and 150 pmol L^{-1}; and (h) styrene: 2, 22, 42, 62 and 82 pmol L^{-1}. The vaporized sample was then gas carried to a glass column containing two adsorbent fibers (pre-concentration system component), with a constant flow (25 mL min^{-1}) of nitrogen (N_2, reagent grade, Praxair, Portugal) controlled by a mass flow meter (Sigma, Germany). The adsorbent fibers are constituted by a glass fiber epoxy glued around a stainless steel wire (60 mm × 0.72 mm o.d.). The packing material consisted of Carboxen 1000 (mesh size 60/80) and polydimethylsiloxane (PDMS). After remaining 20 min in the pre-concentration compartment, i.e., a glass column containing the adsorbent fibers, the analytes were thermally desorbed at 250 °C. The temperature at the glass column was controlled by a coiled tape (Cole Parmer, Illinois, USA). After desorption, the analytes flowed to the separation component, which included a miniaturized column (70 mm × 4 mm o.d.) using PDMS for the active coating matrix. This miniaturized column was placed inside a chamber where temperature control was programmed: the initial temperature was set at 50 °C, followed by a temperature increase at a rate of 10 °C min^{-1} until 100 °C, and 30 °C min^{-1} until 250 °C. Finally, the analytes were carried by the constant flow of N_2 to the detection system component, that is, to the cylindrical tube containing the sensor head. The detection system component used in this study was based on the OF sensor reported elsewhere [31]. The sensor head was implemented onto a 15 mm OF pigtail (9/125 μm core and cladding diameters, respectively) covered by a nanometric film, 2 nm thick, as estimated by Rutherford backscattering spectrometry (RBS), of a siloxane polymer. The OF surface was firstly mechanically uncladded, then cleaned with dichloromethane, and finally cleaved to a length of 15 mm with a precision fiber cleaver (Cleaver V6, Future Instrument, Barcelona, Spain). The OF sensor head integrates the terminal

Table 1
Analytical parameters obtained during the study of the OF device performance for determination of volatile organic compounds.

	Retention time (min)	Linear range (pmol L^{-1})	Linear equation ($y = bx + a$)	Correlation coefficient (R^2)	Residual standard deviation (pmol L^{-1})	Detection limit (pmol L^{-1})
Ethane	1.3	100–500	$y = 0.042x - 3.48$	0.9997 ($p < 1.84 \times 10^{-6}$)	0.125	9.0
Pentane	3.8	50–450	$y = 0.058x - 2.23$	0.9998 ($p < 1.57 \times 10^{-6}$)	0.164	8.5
Heptane	5.4	5–25	$y = 0.608x - 2.09$	0.9992 ($p < 1.00 \times 10^{-5}$)	0.159	0.8
Octane	5.9	2–62	$y = 0.736x + 0.124$	0.9994 ($p < 6.23 \times 10^{-6}$)	0.501	2.0
Decane	6.5	5–425	$y = 0.114x - 0.24$	0.9997 ($p < 1.94 \times 10^{-6}$)	0.359	9.5
Benzene	5.1	10–130	$y = 0.357x - 2.09$	0.9997 ($p < 2.18 \times 10^{-6}$)	0.337	2.8
Toluene	5.7	30–150	$y = 0.376x - 10.49$	0.9998 ($p < 1.13 \times 10^{-6}$)	0.285	2.3
Styrene	6.1	2–82	$y = 0.516x + 1.04$	0.9998 ($p < 8.44 \times 10^{-7}$)	0.237	1.9

arm of a Y coupler (50:50, from Oz Optics, Ottawa, Canada), which has the same core/cladding dimensions of the fiber in order to minimize insertion losses. A sensitive film of poly[methyl(3,3,3-trifluoropropyl) siloxane] (PMTFPS) was deposited on the cleaved OF end by a spray technique, using a coating solution of PMTFPS at 0.01% in dichloromethane. After curing at 70 °C for 12 h, the sensitized OF section was inserted through a Teflon plug into a cylindrical glass tube, where the contact between the sensitive region of the optical system and the volatile analytes of interest took place. The variations in dB in optical power were detected as a result of changes induced by the analyte molecules in the refractive index of the sensitive polymeric film. The reversible changes in the reflected optical power were proportional to the amount of analyte present at the sensitive region. The light conducted through the fiber was generated at a laser diode (Oz Optics, Ottawa, Canada) set at a wavelength of 1550 nm, with the modulated signal detected by the photodetector (Oz Optics, Ottawa, Canada). The optical equipment (laser and detector) was cleaned using a suitable cleaning kit, purchased from AMS Technologies (Barcelona, Spain). The detection system component was kept at laboratory temperature (22 ± 1 °C) and data acquisition was performed on a laptop with home-made software.

The materials for connecting different elements such as valves, fittings, tubes, and sample containers played an important role in obtaining reliable breath samples and reducing potential interferences; the connection between sensor components and sampling tubes was made of Teflon, while the column, tubes and sampling bulb were made of glass. The choice of these materials for the implementation of the OF analytical systems was based on the adequate optical and physical properties of Teflon and glass.To ensure that no gas leakages occured throughout the system, and therefore ensure an adequate analytical performance during the analysis period, the sensor system was regularly checked for leaks, using a spray (Leak-Tec®, Supelco, Bellefonte, USA). Additionally, the operational conditions of links, o-rings, seals and septa were also verified and appropriately replaced when necessary. The sampling bulb was previously purged with pure N_2 (99.999%, Praxair, Portugal) in order to reduce ambient air contamination, guaranteeing an airtight collecting system, free from chemicals and microorganisms from the ambient air [39,40].

2.3. Chemicals and standard mixtures preparation

The standards used for calibration experiments were prepared in carbon disulfide, from Sigma and Fluka chemicals, with a purity of at least 98%. Stock solutions of pentane, heptane, octane, decane, benzene, toluene and styrene, respectively, were prepared in 50.0 mL volumetric flasks. Five standards were then prepared by taking appropriate aliquots of the stock solutions and serially diluting them with carbon disulfide in 25.0 mL volumetric flasks. The solutions were mixed in an ultrasonic bath for 15 min and refrigerated at 4 °C. For analysis, 5 μL aliquots of the solutions were injected into the sampling bulb, using a microsyringe (Hamilton, Bonaduz, Switzerland). Ethane gas standard (from Aldrich) used for calibration experiments was appropriately diluted in N_2 (99.999%, Praxair, Portugal) in order to obtain the desired concentration level and then injected with a 5 μL gastight microsyringe (Hamilton, Bonaduz, Switzerland) into the sampling bulb.

2.4. Breath sampling/collection

Prior to the investigation, subjects were asked to refrain from eating and strenuous physical activity for at least three h prior to the collecting of breath samples. After a 10 min rest in a room next to the laboratory, they were instructed to exhale through a disposable mouthpiece (28 mm × 60 mm, medical express, Penafiel, Portugal) connected to a Teflon plug in a 50 cm long tube, also of Teflon, which was connected to the 3-way valve linked to the sampling bulb (150 mL). The sampling bulb was fitted with a valve system to allow air to pass through during expiration but prevent

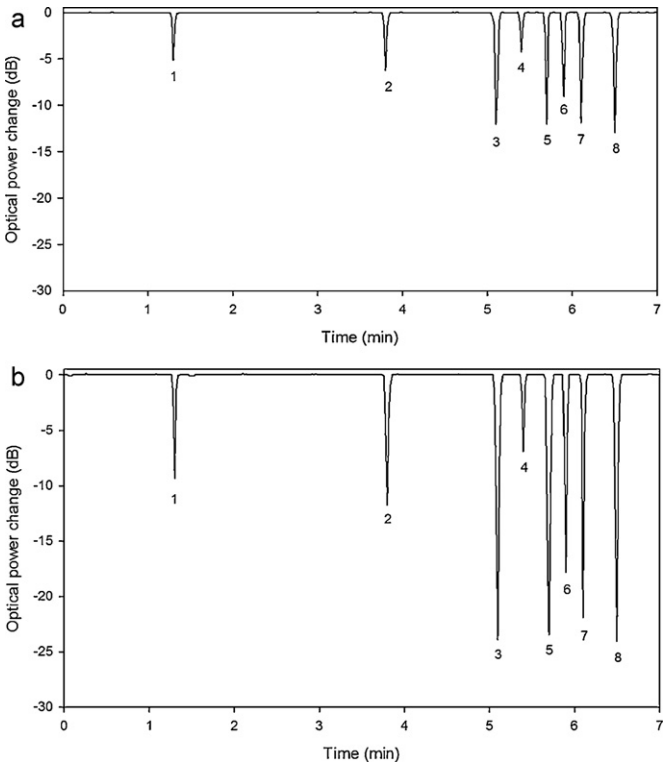

Fig. 2. Analytical response obtained during the analysis of volatile organic compounds by OF sensor: (a) standard mixture of 200 pmol L^{-1} of ethane (1); 150 pmol L^{-1} of pentane (2); 40 pmol L^{-1} of benzene (3); 10 pmol L^{-1} of heptane (4); 60 pmol L^{-1} of toluene (5); 12 pmol L^{-1} of octane (6); 22 pmol L^{-1} of styrene (7); and 125 pmol L^{-1} of decane (8); and (b) standard mixture of 300 pmol L^{-1} of ethane (1); 250 pmol L^{-1} of pentane (2); 70 pmol L^{-1} of benzene (3); 15 pmol L^{-1} of heptane (4); 90 pmol L^{-1} of toluene (5); 22 pmol L^{-1} of octane (6); 42 pmol L^{-1} of styrene (7); and 225 pmol L^{-1} of decane (8).

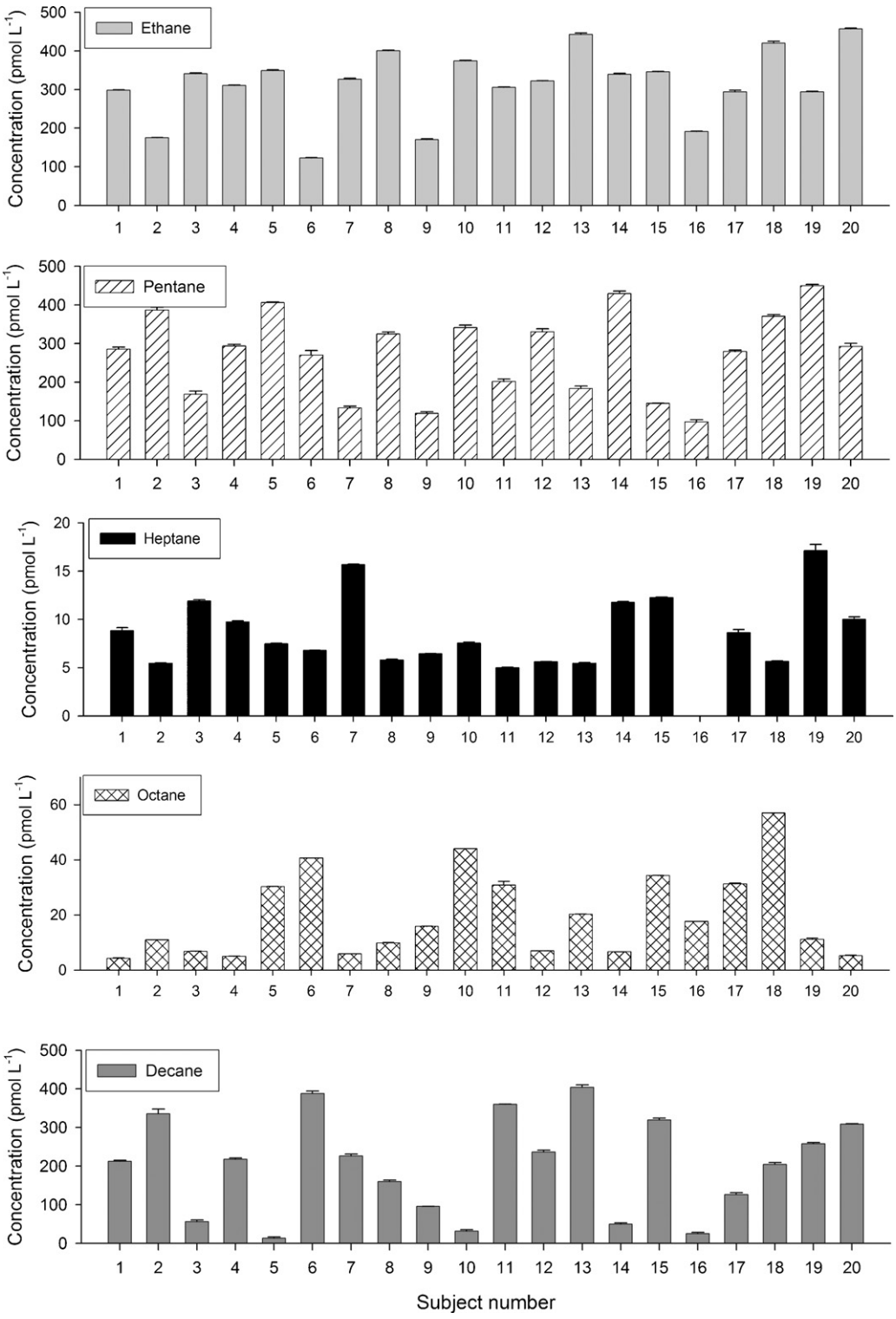

Fig. 3. Concentrations of alkanes exhaled in the breath of 20 healthy subjects.

mixing with ambient air. A new disposable mouthpiece was used for each subject.

The target breathing consisted of a deep inspiration and a 5-s breath-hold, followed by slow and complete exhalation in 10 s. Breath air was discarded during the first 2 s of exhalation and then directed into the sensor system. Five breath samples were collected, together with one sample of room air, for each subject tested. All measurements were performed inside a laboratory under controlled conditions; the central ventilation and temperature control systems were not disturbed by disinfectant dispensers or frequent person traffic.

There are large amounts of water vapor in human breath. The condensation of water vapor in the collecting apparatus may deplete VOCs that are soluble in water and result in falsely depressed concentrations of some analytes [41,42]. Before engaging in the analysis, the potential impact of humidity in the exhaled breath on the sensor analytical performance was assessed by adding 50 mL of ultrapure water to a calibration sample; the pairs

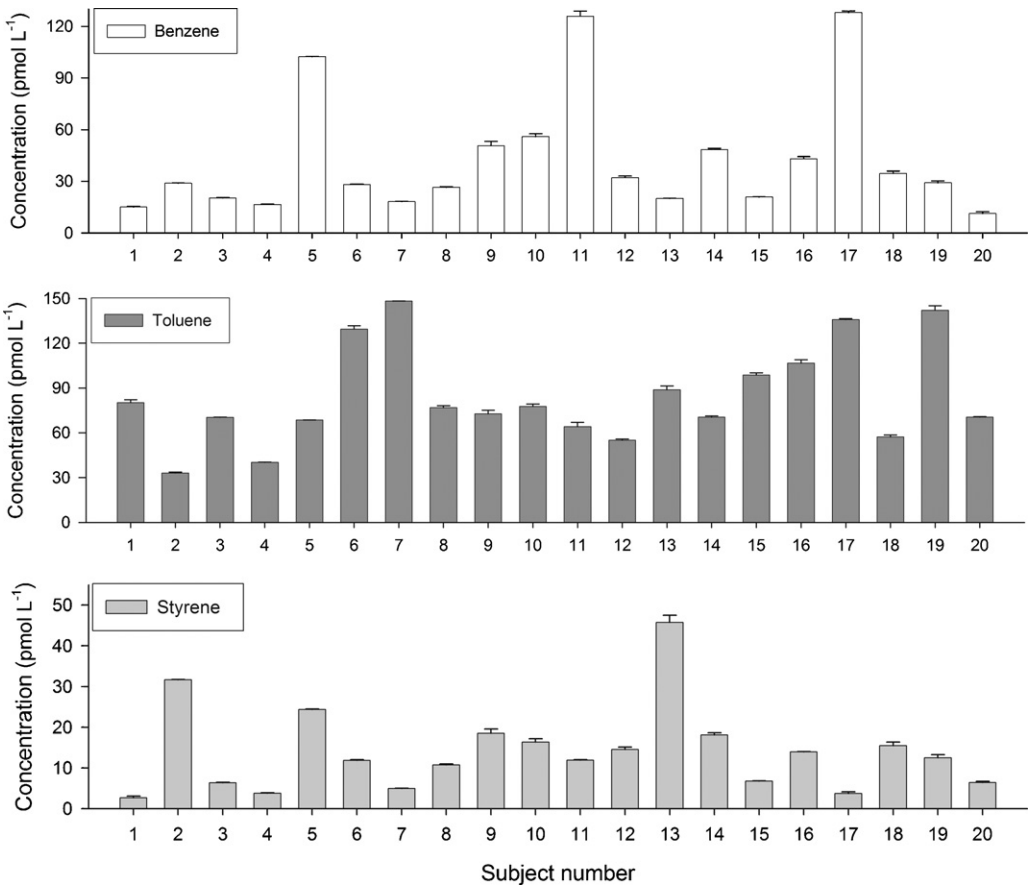

Fig. 4. Concentrations of aromatics exhaled in the breath of 20 subjects.

of humidified and dry standard mixtures were then injected in the sensor system, showing identical retention times and no peak amplitude variation in the humidified mixture.

2.5. GC–MS methodology and associated SPME procedure

The VOCs standards and breath samples were analyzed on a Shimadzu GC-17A GC coupled to a Shimadzu QP5000 GC–MS. The volatile compounds were extracted by solid phase microextraction (SPME), using a 75 μm Carboxen/PDMS fiber (Supelco), which was introduced into the sampling bulb, for 20 min at laboratory temperature, and then thermally desorbed in GC injection port at 250 °C. The VOCs were separated on an Equity-1 column (30 m, 0.25 mm i.d., 1.0 μm film, Supelco), the column was kept isothermal at 60 °C for 5 min, then heated to 200 °C at a rate of 5 °C min^{-1} and, finally, maintained at 200 °C for 20 min. The GC column pressure was 50 KPa. Both the GC–MS methodology and the associated SPME procedure used in this study were based on the method implemented by Poli et al. [11] for breath analysis.

3. Results and discussion

The methodology here developed based on an OF sensing device was first tested for calibration, injecting different concentrations

Table 2
Biomarkers analyzed, their physiological symptoms, and concentration levels found in healthy subjects/control group.

Biomarkers	Metabolic disorders/diseases	Refs.	Concentration found in healthy subjects	Refs.
Alkanes				
Ethane	Vitamin E deficiency in children, lipid peroxidation, oxidative stress.	[7]	0.88 ± 0.09 ppb	[46]
			3.1 ± 0.5 ppb	[47]
Pentane	Peroxidation of lipids, liver diseases, schizophrenia, breast cancer, rheumatoid arthritis	[7]	(107.7–462.7 pM)	[11]
Heptane	Lipid peroxidation, lung cancer, oxidative stress, airway inflammation.	[7]	(5.0–15.3 pM)	[11]
Octane			(4.0–50.8 pM)	[11]
Decane			(14.3–405.5 pM)	[11]
			(1.53–18.14 ng mL^{-1})	[3]
Aromatic compounds				
Benzene	Lipid peroxidation, lung cancer, oxidative stress, airway inflammation.	[7]	(27.7–68.6 pM)	[11]
			(1.02–38.1 ng mL^{-1})	[3]
Toluene			(58.9–140.0 pM)	[11]
Styrene			(5.3–21.8 pM)	[11]
			(1.53–18.14 ng mL^{-1})	[3]

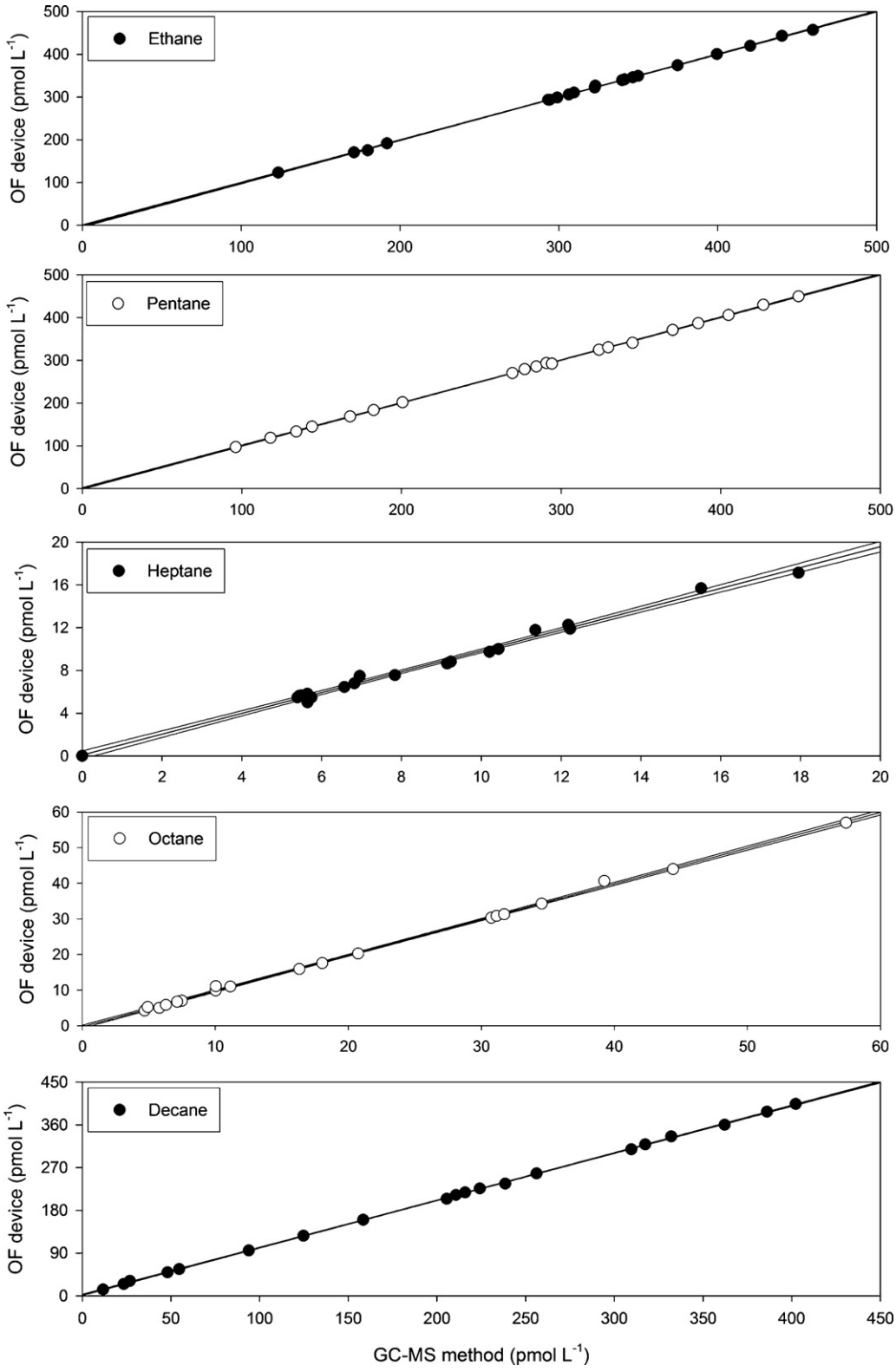

Fig. 5. Comparison of the results obtained during alkanes determination in breath samples by OF device and GC–MS method.

of volatile organic compounds in the sampling bulb. The performance of the OF device for the determination of target analytes was evaluated by studying various analytical parameters, such as linearity, sensitivity and detection limit. The results obtained in this set of experiments are shown in Table 1 and Fig. 2 shows the analytical response profile for standard mixtures with different concentrations of volatile organic compounds (alkanes and aromatic compounds).

The OF device shows a high statistical degree of linearity for the calibration model used ($y = bx + a$) for volatile organic compound detection, as it can be inferred from the R^2 and respective p values shown in Table 1.

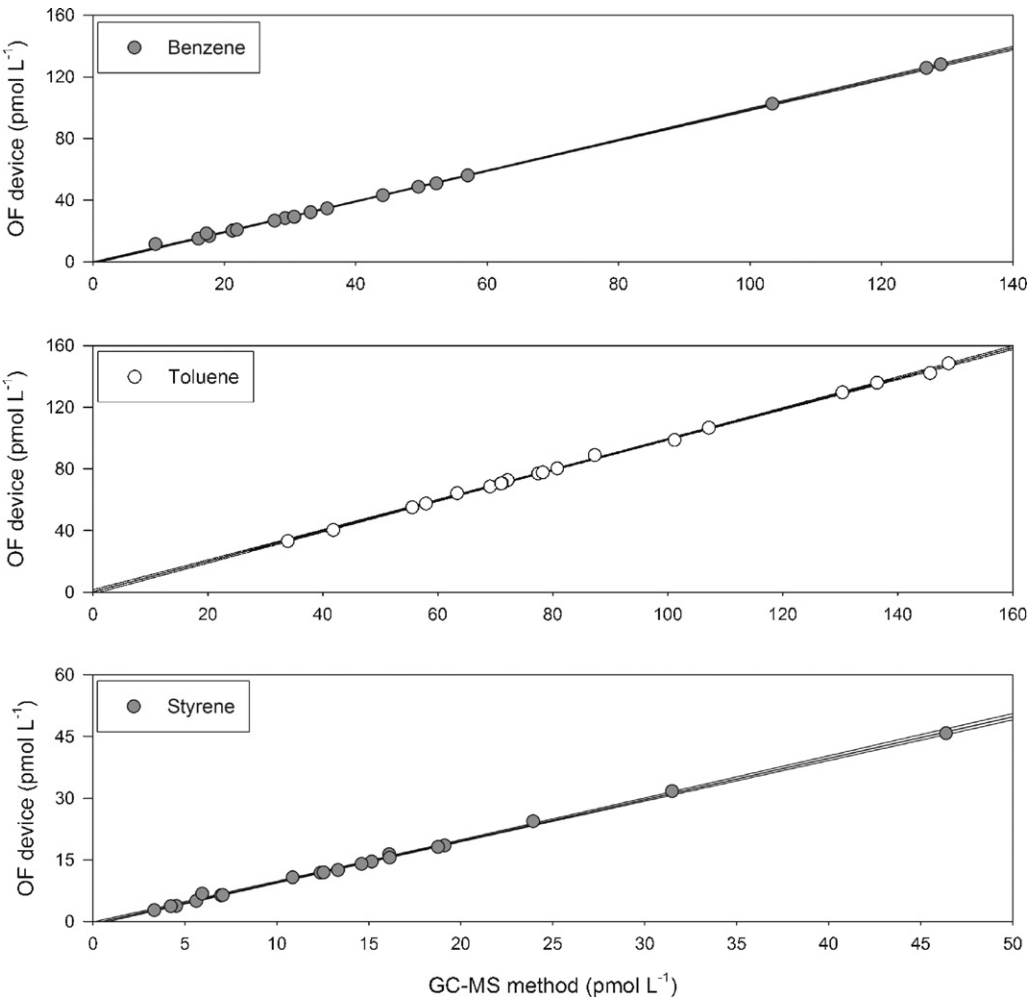

Fig. 6. Comparison of the results obtained during aromatic compounds determination in breath samples by OF device and GC–MS method.

The detection limits, calculated on the basis of three times the residual standard deviation [26,27,43], were of the order of magnitude of pmol L^{-1} for all the organics under study, varying from 0.8 pmol L^{-1} for heptane to 9.5 pmol L^{-1} for decane. For the analysis performed by GC–MS, the detection limits were found to be 14.3 pmol L^{-1} for ethane; 11.6 pmol L^{-1} for pentane; 0.8 pmol L^{-1} for heptane; 2.0 pmol L^{-1} for octane; 14.1 pmol L^{-1} for decane; 3.4 pmol L^{-1} for toluene; 5.0 pmol L^{-1} for benzene; and 3.6 pmol L^{-1} for styrene.

From the calibration study, the analytical performance of the OF sensor was found to decrease for concentration values much lower than or over the linear range (Table 1).

Taking into account this sensor's non-linear response for concentrations lower than the smallest concentration value of the calibration curve, the detection limit for the OF sensor was recalculated based on the SDS calibration model proposed by Silva et al. [44]. This calibration model considers the entire range of the analytical response of the OF sensor, comparing more advantageously with a linear calibration model. The detection limit values established according to this model are slightly lower than the first point of the calibration curves, i.e., 100 pmol L^{-1} for ethane; 50 pmol L^{-1} for pentane; 5 pmol L^{-1} for heptane; 2 pmol L^{-1} for octane; 5 pmol L^{-1} for decane; 10 pmol L^{-1} for benzene; 30 pmol L^{-1} for toluene; and 2 pmol L^{-1} for styrene.

The total analytical time was 10 min for the analysis performed by OF sensor, and the individual retention times for each compound are shown in Table 1. Although they are not comparable due to different separation conditions, in the GC–MS method the individual retention times obtained were: 7.5 min for ethane; 24.8 min for pentane; 35.1 min for benzene; 37.3 min for heptane; 41.1 min for toluene; 43.2 min for octane; 45.3 min for styrene; and 47.1 min for decane.

Fig. 3 shows the levels of alkanes found in the exhaled air of 20 subjects, determined by the OF sensor. The ethane concentration varied from 123 to 457 pmol L^{-1} in the breath analysis performed. The breath samples analyzed showed an average concentration level of pentane of 257 pmol L^{-1}. The maximum occurrence of heptane was around 17.1 pmol L^{-1}, and octane and decane average mean concentrations were found to be 19.8 and 201.3 pmol L^{-1}, respectively.

Fig. 4 shows the results obtained for the aromatic compounds in the breath samples analyzed from the same 20 subjects. Toluene is the most abundant aromatic compound analyzed in comparison with benzene and styrene concentrations, which varied from 11.4 to 128.0 pmol L^{-1} for benzene and from 2.7 to 15.7 pmol L^{-1} for styrene.

The VOCs profile, including alkanes and aromatic hydrocarbons in human breath, varies significant between subjects, both qualitatively and quantitatively [1]. The levels of these compounds in human breath can range from 10^{-12} ppt to 10^{-9} ppb [45]. In the breath samples analyzed, the levels of the exhaled target compounds correlate to those already reported for these compounds in healthy subject's breath [11], from a comparison to the range of concentration shown in Table 2. Table 2 also shows a list of the

Table 3
Biomarkers analyzed, metabolic disorder/disease, and concentration levels found in non-healthy subjects.

Biomarkers	Concentration found in non-healthy subjects	Refs.	Metabolic disorders/diseases
Alkanes			
Ethane	64–2160 (ppb)	[49]	Lung cancer
Pentane	1–3 (ppb)	[49]	Lung cancer
	361.3–1112.5 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer
Heptane	1.5–34.0 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer
Octane	22.4–112.9 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer
Decane	277.9–1321.6 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer
Aromatic compounds			
Benzene	0.9–6.6 (ppb)	[49]	Lung cancer
	62.2–132.2 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer
Toluene	1.9–6.4 (ppb)	[49]	Lung cancer
	118.7–237.5 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer
Styrene	8.5–37.2 ($\times 10^{-12}$ M)	[11]	Patients with non-small cells lung cancer

biomarkers analyzed, their related diseases, and metabolic disorders.

Figs. 5 and 6 show the comparison of the results obtained for breath sample analysis by OF sensor and GC–MS method. The analysis of the results reported in these figures suggests that: (a) a linear correlation can be established between the two analytical methodologies for the eight volatile compounds analyzed, with correlation coefficients (R^2) of 0.9997 ($p < 3.11 \times 10^{-33}$) for ethane, 0.9998 ($p < 7.13 \times 10^{-35}$) for pentane, 0.9925 ($p < 1.40 \times 10^{-20}$) for heptane, 0.9988 ($p < 8.26 \times 10^{-28}$) for octane, 0.9999 ($p < 5.91 \times 10^{-36}$) for decane, 0.9995 ($p < 6.74 \times 10^{-31}$) for benzene, 0.9991 ($p < 1.05 \times 10^{-28}$) for toluene, and 0.9981 ($p < 5.50 \times 10^{-26}$) for styrene; (b) for all evaluated analytes in the exhaled air, the regression line has not significantly different intercept of zero and a not significantly different slope of 1, supporting the hypothesis that the results obtained with the two analytical method cannot be statistically differentiated; (c) narrow intervals at 95% confidence level were observed, which suggest a low dispersion levels of the results obtained by the two applied analytical methodologies on breath analysis.

The comparison of the results obtained for volatile compounds analysis in breath samples, by the OF device and GC–MS, showed that the difference in the mean values between the two analytical methodologies is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference ($p = 0.903$, 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) between the two analytical systems implemented for breath analysis. This particular statistical analysis was performed using SigmaStat 3.0 [48], applying a *t*-test on ethane, pentane, heptane, octane, decane, benzene, toluene and styrene results.

In order to ascertain the applicability of the developed OF device for breath analysis and its usefulness in clinical context, the potential interference of 2-propanol in the breath analysis was evaluated. This compound is widely use as a disinfectant, i.e., for disinfection of large skin areas of patients. No interference could be found since the 2-propanol produces a peak in a different time window of the target analytes; as a consequence, it is also possible to infer that the OF sensor could be also applied to breath analysis under clinical conditions. The sensor response for carbon disulfide, as this compound was used as solvent for standard mixtures preparation, was also tested but no significant analytical signal was obtained, indicating therefore that carbon disulfide does not interfere with volatile organic analysis.

The OF sensor also showed high potential for application: (a) in cross-sectional studies of breath biomarkers, i.e., a control group of healthy subjects is compared with a patient or diseased group; (b) for disease progression studies, observing the course of a disease within one patient group (Table 3 shows some concentration levels of volatile organic compounds found in non-healthy subjects, reported in the literature); and (c) for occupational exposure of volatile organic compounds.

4. Conclusions

The sensor system developed exhibits an adequate analytical performance in terms of linearity, accuracy, and detection limits for breath analysis.

The reported analytical device allows in-line, and near real-time, analysis of breath samples, keeping the analytical performance at the same level for actual breath samples using the GC–MS reference method. Simple and fast breath sampling, ease of use, and compact design were also analytical features checked for the sensor system. Thus, the proposed optical fiber-based analyzer can constitute an excellent platform for inexpensive and useful clinical testing for the diagnosis of many different diseases.

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References

[1] W. Cao, Y. Duan, Clin. Chem. 52 (2006) 800–811.
[2] T.H. Risby, S.F. Solga, Appl. Phys. B 85 (2006) 421–426.
[3] W. Miekisch, J.K. Schubert, G.F.E. Noeldge-Schomburg, Clin. Chim. Acta 347 (2004) 25–39.
[4] F. Di Francesco, R. Fuoco, M.G. Trivella, A. Ceccarini, Microchem. J. 79 (2005) 405–410.
[5] B. Buszewski, M. Kesy, T. Ligor, A. Amann, Biomed. Chromatogr. 21 (2007) 553–566.
[6] D. Smith, P. Spanel, Analyst 132 (2007) 390–396.
[7] C. Wang, P. Sahay, Sensors 9 (2009) 8230–8262.
[8] H.P. Chan, C. Lewis, P.S. Thomas, Cancers 2 (2010) 32–42.
[9] H.P. Chan, C. Lewis, P.S. Thomas, Lung Cancer 63 (2009) 164–168.
[10] C. Grote, J. Pawliszyn, Anal. Chem. 69 (1997) 587–596.
[11] D. Poli, P. Carbognani, M. Corradi, M. Goldoni, O. Acampa, B. Balbi, L. Bianchi, M. Rusca, A. Mutti, Respir. Res. 6 (71) (2005).
[12] B. Moser, F. Bodrogi, G. Eibl, M. Lechner, J. Rieder, P. Lirk, Physiol. Neurobiol. 145 (2005) 295–300.
[13] G.R. Harrison, A.D. Critchley, C.A. Mayhew, J.M. Thompson, Br. J. Anaesth. 91 (2003) 797–799.
[14] V. Ruzsanyi, J. Baumbach, P. Litterst, M. Westhoff, L. Freitag, J. Chromatogr. A 1084 (2005) 145–151.
[15] A.M. Diskin, P. Spanel, D. Smith, Physiol. Meas. 24 (2003) 107–120.

[16] C. Turner, P. Spanel, D. Smith, Physiol. Meas. 27 (2006) 321–337.
[17] D. Smith, P. Spanel, Mass Spectrom. Rev. 24 (2005) 661–700.
[18] H. Lord, Y.F. Yu, A. Segal, J. Pawliszyn, Anal. Chem. 74 (2002) 5650–5657.
[19] W. Ma, X. Liu, J. Pawliszyn, Anal. Bioanal. Chem. 385 (2006) 1398–1408.
[20] M.R. McCurdy, Y. Bakhirkin, G. Wysocki, R. Lewicki, F.K. Tittel, J. Breath Res. 1 (2007) 1–12.
[21] M. Mürtz, Opt. Photon. News 16 (2005) 30–35.
[22] M. Fleischer, E. Simon, E. Rumpel, H. Ulmer, M. Harbeck, M. Wandel, C. Fietzek, U. Weimar, H. Meixner, Sens. Actuators B 83 (2002) 245–249.
[23] C.D. Natale, A. Macagnano, E. Martinelli, R. Paolesse, G. D'Arcangelo, C. Roscioni, A. Finazzi-Agro, A. D'Amico, Biosens. Bioelectron. 18 (2003) 1209–1218.
[24] H.H. Huang, J. Zhou, S.Y. Chen, L. Zeng, Y.P. Huang, Sens. Actuators B 101 (2004) 316–321.
[25] O.S. Wolfbeis, Anal. Chem. 80 (2008) 4269–4283.
[26] L.I.B. Silva, T.A.P. Rocha-Santos, A.C. Duarte, Talanta 76 (2008) 395–399.
[27] L.I.B. Silva, T.A.P. Rocha-Santos, A.C. Duarte, Sens. Actuators B 132 (2008) 280–289.
[28] L.I.B. Silva, T.A.P. Rocha-Santos, A.C. Duarte, Global Nest J. 10 (2008) 217–225.
[29] L.I.B. Silva, A.M. Costa, A.C. Freitas, T.A.P. Rocha-Santos, A.C. Duarte, Int. J. Environ. Anal. Chem. 89 (2009) 183–197.
[30] L.I.B. Silva, A.V. Panteleitchouk, A.C. Freitas, T.A.P. Rocha-Santos, A.C. Duarte, Anal. Methods 1 (2009) 100–107.
[31] L.I.B. Silva, T.A.P. Rocha-Santos, A.C. Duarte, Talanta 78 (2009) 548–552.
[32] L.I.B. Silva, F.D.P. Ferreira, A.C. Freitas, T.A.P. Rocha-Santos, A.C. Duarte, Talanta 80 (2009) 853–857.
[33] F.D.P. Ferreira, L.I.B. Silva, A.C. Freitas, T.A.P. Rocha-Santos, A.C. Duarte, J. Chromatogr. A 1216 (2009) 7049–7054.
[34] L.I.B. Silva, F.D.P. Ferreira, A.C. Freitas, T.A.P. Rocha-Santos, A.C. Duarte, Food Chem. 123 (2010) 806–813.
[35] O.S. Wolfbeis, H.E. Posch, H.W. Kroneis, Anal. Chem. 57 (1985) 2556–2561.
[36] A. Mills, A. Lepre, L. Wild, Sens. Actuators B 38–39 (1997) 419–425.
[37] S. DuBois, S. Eng, R. Bhattacharya, S. Rulyak, T. Hubbard, D. Putnam, D.J. Kearney, Digest. Dis. Sci. 50 (2005) 1780–1784.
[38] K. Mitsubayashi, T. Minamide, K. Otsuka, H. Kudo, H. Saito, Anal. Chim. Acta 573–574 (2006) 75–80.
[39] W.H. Cheng, W.J. Lee, J. Lab. Clin. Med. 133 (1999) 218–228.
[40] R.A. Glaser, J.E. Arnold, S.A. Shulman, Am. Ind. Hyg. Assoc. J. 51 (1990) 139–150.
[41] M. Phillips, Anal. Biochem. 247 (1997) 272–278.
[42] M. Phillips, J. Greenberg, Clin. Chem. 38 (1992) 60–65.
[43] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, fifth ed., Pearson Prentice Hall, New York, 2005.
[44] L.I.B. Silva, M. Freitas, T.A.P. Rocha-Santos, A.C. Duarte, Talanta 82 (2010) 1403–1411.
[45] H. Yu, L. Xu, P. Wang, J. Chromatogr. B 826 (2005) 69–74.
[46] P. Paredi, S.A. Kharitonov, P.J. Barnes, Am. J. Respir. Crit. Care Med. 162 (2000) 1450–1454.
[47] J.E. Abela, K.D. Skeldon, R.C. Stuart, M.J. Padgett, Biosci. Trends 3 (2009) 110–114.
[48] SigmaStat 3.0 (Statistic Software for Windows), Jandel Scientific, Erkrath, Germany, 1994.
[49] R.F. Machado, D. Laskowski, O. Deffenderfer, T. Burch, S. Zheng, P.J. Mazzone, T. Mekhail, C. Jennings, J.K. Stoller, J. Pyle, J. Duncan, R.A. Dweik, S. C. Am. J. Respir. Crit. Care Med. 171 (2005) 1286–1291.