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Review

Breath air analysis and its use as a biomarker in biological monitoring of occupational and environmental exposure to chemical agents

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Abstract

The analysis of exhaled air has several advantages since it is a noninvasive method applicable to a large number of toxic agents, in addition to being a simpler matrix than those of other biological samples such as urine and blood. However, it presents some challenges, such as the necessity of a more sensitive sampling procedure, since the chemical substances eliminated through exhaled air are unchanged in form, not being metabolized, and exhaled compounds are present at extremely low concentrations, i.e. in the nanomolar range. To improve the sensitivity and precision of measurement of the concentration of these substances in exhaled air, the sample usually has to be concentrated before assay by gas chromatography. To this end, the use of the solid-phase microextraction (SPME) technique has been proposed as an efficient sampling method. This paper presents a revision of breath analysis as a biomarker for occupational and environmental exposure to chemicals. The sampling methods and the potential use of SPME for determining chemical substances in exhaled air are discussed.

Keywords: Breath analysis; Gas chromatography; Solid-phase microextraction; Biomarker; Occupational exposure; Environmental exposure

Contents

1. Introduction

In the environmental and occupational health areas, there is great interest in the contribution of analytical chemistry for the determination of toxic substances in the evaluation of risk to human health. Breath analysis has been used in occupational medicine since 1930, when studies evaluating human exposure to volatile organic compounds (VOC) were first reported [1,2]. The development of more sensitive analysis methods with new extraction and detection techniques permits the determination of chemical substances in trace amounts in exhaled air and has contributed to the increase in breath analysis as a biomarker in monitoring of occupational and environmental biological exposure to several volatile chemical agents.

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From the occupational viewpoint, VOC are important because of the large-scale production, their use in manufacturing processes and their high toxic potential. These substances target the central nervous system and are easily absorbed by the human organism through the lungs and, in some cases, through the skin.

The health risks resulting from occupational exposure to these chemical agents require the attention of Governments and the companies that produce or use these substances in their industrial processes and who are responsible for controlling exposure to them. Occupational or environmental exposure to chemicals may induce various diseases in individuals and populations and may lead to major public health problems. Therefore, monitoring exposure based on biomarkers allows the evaluation of individual and group hazards and the early detection of exposure to hazardous chemicals, thus significantly reducing their effects on health.

Correlation of environmental chemical agents and disease is a difficult task, considering the complex interaction between the human organism and the environment, particularly when there is a long period between exposure and manifestation of disease [3]. It has not been possible to establish biomarkers for many chemical substances that present a biological interaction with the human organism. Therefore, indicators of biological exposure may allow estimation of the internal dose before the toxic effects are manifested.

The determination of VOC in exhaled air has been used as an exposure biomarker in biological monitoring of occupational and environmental chemical agents since it is a noninvasive method and has a simpler matrix than those of other biological samples such as urine and blood. It also has great clinical interest for the detection of some chemical agents as biomarkers of diseases like lung cancer, liver disease, myocardial infarction and diabetes [4,5].

2. Exposure biomarkes: definition and concepts

The concept of biomarkers has been developed to estimate the relationship between environmental and occupational exposures and their subsequent effects on individuals or on a group. The goal of biomarker research and application is to prevent disease by reducing exposures to hazardous agents through the early identification of exposure and response.

Several biological parameters may be altered as a consequence of the interaction between chemical agents and the organism. However, the quantitative determination of these agents as biological indicators or biomarkers may be used only if there is a correlation between exposure intensity and the biological effect of these substances. As a result, a biomarker is any substance or the product of its biotransformation (exposure biomarker) or any early biochemical change resulting from chemical action (effect biomarker), whose determination may be used in the assessment of exposure or its health risks. Thus, an exposure biomarker estimates the internal dose of the respective chemical agent in the body [6].

Depending on the chemical and the analyzed biological parameter, the term internal dose may cover different concepts. It may mean the amount of chemical recently absorbed, either during the preceding day or during the past months when the chemical has a long biological half-time. The internal dose may also mean the amount of chemical stored in one or in several body compartments or in the whole body as integrated exposure or specific organ dose. Finally, the internal dose means the amount of chemical bound to the critical sites of action. In fact, it is the biologically effective dose, since the critical sites are easily accessible [6,7].

3. Exhaled-air analysis principles

The chemical substances that exist mainly in the gas phase at room temperature are eliminated unchanged, principally through the lungs. According to Henry's law, the amount of a given substance eliminated through the lungs is proportional to its vapor pressure [8].

Breath analysis is based on the equilibrium between alveolar air and pulmonary capillary blood. The compounds present in exhaled breath are proportional to their blood concentrations because of rapid gas exchange at the blood–gas interface in the lungs. No specialized transport systems have been described for the excretion of toxic substances by the lungs. These substances seem to be eliminated by simple diffusion. The elimination of gas is, in general, inversely proportional to its absorption by the lungs [8,9].

This behavior is true for a gas or a vapor that does not have a special affinity for certain blood components. When it is inhaled, gas molecules diffuse from the alveolar space into the blood and then dissolve. The uptake of a gas by a tissue usually involves a simple physical dissolution. This dissolution facilitates the partition of gas molecules between the air and blood during the absorptive phase and between blood and other tissues during the distribution phase.

The chemical substances present in the alveoli remain there sufficiently long to reach equilibrium with the blood. The contact of inhaled gas with blood is continued in the alveoli, and equilibrium occurs easily and quickly. At equilibrium, the ratio of the concentration of the chemical in the blood to that in the gas phase is constant. This is called the blood–gas partition coefficient (K), and it is unique for each gas [8].

The interpretation of biological monitoring of VOC using breath analysis as a biomarker requires that special attention be paid to factors that influence pulmonary excretion. According to Wilson [10] these factors are: ventilation–perfusion, diffusion–adsorption–desorption, metabolism, breathing technique, temperature, blood composition and time [10].

4. Use of exhaled air as an exposure biomarker

Hundreds of VOC are present in human and animal breath. These substances may be generated in the body or absorbed through environmental or occupational exposure [11]. The analysis of chemical substances in exhaled air has been used as a biomarker to assess occupational exposure to industrial solvents and has been the object of several studies that have demonstrated a correlation between VOC levels in exhaled air and exposure to chemical substances at the workplace [12–29].

Egeghy et al. [29] investigated the association of benzene uptake by automobile mechanics exposed to benzene through their contact with gasoline vapor and engine exhaust. They measured benzene in the air and benzene in end-exhaled breath among 81 workers from 12 automobile repair garages. This study indicated strong linear trends between the benzene concentrations in air and breath, but a more random variation was apparent among smokers than non-smokers. The results suggested that benzene in cigarette smoke affected the breath–exposure relationship among smokers. The median concentration of benzene in breath at the beginning of the exposure period was 12.9 $\mu g/m^3$ for non-smokers and 33.3 $\mu g/m^3$ for smokers. This result indicates that the breath levels of benzene in smokers prior to the work shift were dictaded largely by their smoking habits [29].

Using SPME and GC–MS, Guidotti et al. [27] determined the levels of chlorinated solvents in exhaled air of subjects exposed in the workplace. They analyzed five chlorinated solvents: chloroform, trichloroethylene, tetrachloroethylene, tetrachloroethane and carbon tetrachloride. The theorical detection limits (TDL) were 0.5–5 ppb, which show the high sensitivity of their method. The authors considered it suitable for monitoring workers exposed to low concentrations of these chemicals [27]. This TDL was similar to that (1.5 ng/L) determinated by Plebani et al. [15], whose methods have been successfully applied to biological monitoring [15].

Chen et al. [19] evaluated the relationship between the breath concentrations of, and personal exposure to, toluene, xylene and ethylbenzene for 30 workers from gasoline stations. Each worker provided a sample of exhaled breath after his personal exposure air was sampled. They found that breath concentrations of toluene and xylene were significantly correlated with personal monitoring concentrations. Exhaled ethylbenzene levels were too low to present a relationship between concentration and personal exposure levels. In summary, the exhaled toluene, xylene and ethylbenzene concentrations ranged from 4.3 to 41.8, 0.9 to 13.9, and 0.2 to 6.5 ppb, respectively. The results showed that exhaled breath is suitable for use as a biological exposure index even at the ppb level. However, some gasoline service workers were exposed to high levels of analyzed VOC [19].

Egeghy et al. [20] measured benzene and naphthalene in air and breath to estimate exposures to these substances among military personnel working with jet fuel. They investigated subjects who had been assigned a priori into low, moderate and high exposure categories. All subjects provided samples of end-exhaled air at the beginning and end of the monitoring period. These samples are designated as "pre and post-exposure" breath samples. Benzene and naphthalene exposures differed significantly among the three exposure categories. The median breath concentrations for persons in the low, moderate and high exposure categories were 4.6, 9.0, and 11.4 μg benzene/m^3; 0.73, 0.93, and 1.83 μg naphthalene/m^3, of breath. In addition, concentrations of both benzene and naphthalene were significantly higher in "post-exposure" than in "pre-exposure" breath. The levels in "post-exposure" breath were significantly different between the high and low, and high and moderate categories, but the difference between the moderate and low categories was not significantly for either compound. The exposure–breath relationships were weak for both benzene and naphthalene in the low and moderate exposure categories. However, the corresponding relationships were much stronger in the high exposure category. Air naphthalene correlated highly with a priori categories of jet fuel exposure and, unlike benzene, was not unduly influenced by background sources and cigarette smoking [20].

Ghittori et al. [22] described a method for the determination of toluene in exhaled air as a biomarker of occupational exposure. The correlation coefficient (r) for the correlation of breath analysis with environmental toluene level was 0.822. The concentration of toluene in alveolar air ranged from 159 to 3354 ng/L. The breath analysis permitted the collection of data on the elimination of toluene by this pathway [22].

Sweet et al. [23] developed of a method for the determination of perchloroethylene in breath. They evaluated the sensitivity, specificity, precision, and speed of analysis for the perchloroethylene method. Measurements made for field samples were in the range of 0.9–6.0 ppm with a coefficient of variation for all measurements (three replicates) equal to 5.8% [23].

Despite the relatively recent and still limited application of exhaled air as a biomarker in the environmental health field, several studies have demonstrated its potential in the evaluation of populations exposed to environmental chemical agents [24,30,31]. Perbellini et al. [2] demonstrated that the concentration of 1,3-butadiene, 2,5-dimethylfuran and benzene may be detected and measured in the exhaled air of individuals environmentally exposed to these substances. The analysis of exhaled air presented a good correlation with the levels of these substances in the blood and urine in this study. The median 1,3-butadiene, 2,5-dimethylfuran and benzene levels in alveolar air were 1.2 ng/L, 0.5 ng/L, and 5.7 ng/L, respectively [2].

Breath analysis has a promise for identifying important chemicals and routes of environmental exposure, such as cigarette smoking, which is the largest single source of exposure to many toxic substances Yu and Weisel [32] determined the concentration of benzene in breath after exposure to environmental benzene. Five volunteers were exposed to environmental tobacco smoke at different exposure levels and different exposure durations. Benzene in breath was confirmed as a short-term biomarker for environmental benzene exposure at the sub-ppm level [32].

In another study, benzene levels in human breath and in ambient air were compared in an urban area and in a more remote coastal area. Ambient benzene levels were seven-fold higher in the urban area than in the coastal area. In the urban area, benzene concentrations in smokers' breath (6.8 ppb) was higher than in nonsmokers' breath (2.5 ppb), and both were higher than the respective ambient air levels (3.3 and 1.4 ppb). The same patterns held true for the breath levels found in subjects from coastal areas, where breath levels of benzene were again higher in smokers (4.8 ppb) than in nonsmokers (1.3 ppb), and both were higher than benzene levels in ambient air (0.23 ppb) [33]. Jo and Pack [34] analyzed exhaled air to estimate the environmental exposure to benzene associated with cigarette smoking as the single most important source of this carcinogenic agent [34]. Gordon et al. [35] proposed VOC as breath biomarkers

for active and passive smoking. Their findings were useful in models of environmental tobacco smoke exposure and risk [35].

In addition to the analysis of exhaled air for evaluation of occupational and environmental exposure to VOC, the presence of several of these substances in exhaled air has great clinical importance. Recently, increased interest in these compounds encountered in exhaled air resulted from the identification of correlations with a number of illnesses. The annual number of publications dealing with diagnostic breath tests has steadily risen, especially over the last few years [36–41].

The use of the exhaled air analysis in the diagnosis of metabolic disorders and diseases was established for the determination of acetone in the study of diabetes [42], a chronic disease in which the pancreas is unable to produce the insulin necessary for sugar metabolism. The analysis of several aliphatic and aromatic hydrocarbons in exhaled air has been studied in an attempt to identify potential lung cancer tumor markers in affected populations and control groups [39–41,43,44].

In addition, the following markers have been used: alkanes to evaluate lipid metabolism, carbon dioxide for respiratory disturbances, dimethylamine for the diagnosis of kidney disorders, nitrogen oxide as a marker of asthma, in addition to other markers [45]. Furthermore, the analysis of alcohol in exhaled air to determine whether drivers are under the influence of alcohol was one of the first and is probably the most common application of exhaled air analysis among the many methods described [46].

The determination of chemical substances in exhaled air offers some advantages because of its selectivity and sensitivity in the estimation of recent exposure. Since it is a noninvasive sampling method, it is promptly accepted by workers. In addition, its matrix (air) is extremely simple compared to other biological fluids and much easier to analyze. In general, the method may be applied to a relatively large number of toxic substances, all of which are of interest to occupational and environmental health.

Although the toxic kinetics of a large number of these substances is known, the relationship between the concentration in exhaled air and exposure is not well known, especially because of the environmental fluctuation that is common in the workplace [18]. The analysis of exhaled air also has the disadvantage that only a few of the substances that cross the alveolar capillary membrane are present in the environment and frequently exist in low concentrations so a technique with high analytical sensitivity is required.

Nevertheless, many studies of exposed and unexposed populations have been performed to establish the relationship between breath analysis and environmental analysis in the workplace, in addition to the development analytical methods and techniques that allow detection of low levels of these chemical agents in human exhaled air [47,48]. Table 1 lists the most recent publications on occupational and environmental exposure to chemical substances and their determination in exhaled air.

Table 1
Recent publications on the analysis of exhaled air and VOC exposure biomarkers

Chemical substance	Reference
Benzene; toluene; xylene; phenol	Moser et al. [37]
Perchloroethylene	Sweet et al. [23]
Toluene	Yoshida et al. [21]
Benzene; naphthalene	Egeghy et al. [20]
1,3-Butadiene; 2,5-dimethylfuran; benzene	Perbellini et al. [2]
Toluene; xylene; ethyl benzene	Chen et al. [19]
Xylene; trimethylbenzene	Wilson et al. [49]
Benzene	Egeghy et al. [29]
Benzene	Gordon et al. [35]
Benzene; toluene, xylene; ethyl benzene	Jo and Kim [17]
Benzene; toluene; hexane; trimethylbenzene; methylene chloride	Thrall et al. [18]
Benzene	Jo and Pack [34]
Xylene; toluene	Jones et al [50]

5. Biological exposure limits

Biological exposure limits (BEL) are reference values intended as guidelines for the evaluation of potencial health hazards. BEL is defined as "the maximum permissible quantity" of a biomarker and does not indicate a sharp distinction between hazardous and non-hazardous exposure. According to current knowledge, these conditions generally do not impair the health of the employee, even if exposure is repeated or of long duration. The BEL reflect different philosophies depending on regulatory authority and country. It may be health-based, such as the "Biologische Arbeitstoffoleranzwerte" (BAT) of Germany, or related to an equivalent hygiene limit such as the "Biological Exposure Index" (BEI) of The American Conference of Governmental Industrial Hygienists (ACGIH)-USA [6]. Although no BAT exists for exhaled air, the BEI list includes four substances (ethylbenzene, methylchloroform, perchloroethylene and trichloroethylene). However, BEI values exist only for two of them (methylchloroform −40 ppm and perchloroethylene −5 ppm) [51].

The interpretation of analytical data is a critical point in defining the BEL of chemical substances in exhaled air. There are limited data for determining biological guidance values for many substances that could be used for breath monitoring. The use of exhaled breath as an occupational biomarker tool is still not widely used. It needs more studies to establish the pattern about exposure and non-exposure population.

6. Sampling techniques and breath analysis

There are many different sampling techniques for breath analysis. Several containers are used to sample exhaled air, such as glass tubes and plastic bags, from which a sub-sample is transferred directly to the analysis system by means of syringes, or solid adsorbants, from which the components are thermally desorbed [12,52]. This is a time-consuming process consisting of a number of steps that may lead to loss of compounds. Compounds may also be adsorbed onto the surface of the containers.

Therefore, several sampling devices [23,28,29,35,41] have been proposed to guarantee efficient sampling that will truly represent the content of exhaled air during the exhalation time. The device must necessarily have a safe storage system to prevent the loss of the analyte between sampling and the analysis itself. Another technique consists of the use of solid adsorbants without

a sampling container, such as in the solid-phase microextraction (SPME) technique [53]. Considered a promising technique for gas chromatography, it will be dealt with separately in more detail.

The analysis techniques most commonly used to determine a chemical substance in exhaled air are gas chromatography with detection by flame ionization (FID), mass spectrometry (MS) and flame photometry (FPD), and, more recently, mass spectrometry using chemical ionization such as proton-transfer-reaction mass spectrometry (PTR-MS), selected ion flow tube-mass spectrometry (SIFT-MS) or ion-molecule reactions (IMR) [37,38]. The development of more sensitive and specific detectors has facilitated the determination of very low concentrations of chemical agents in exhaled air and has helped to overcome the main limitation of this biomarker.

The definition of some factors such as: (1) the best sampling moment, (2) the type of exhalation, and (3) the sampling technique for an adequate validation of breath analysis as a biomarker of VOC is fundamental. Exhaled breath has two components. The first 150 mL of breath, called "dead-space air", comes from the trachea and the bronchioles where no gaseous exchange between the blood and air occurs. The remaining 350 mL is called "alveolar breath". It comes from deep within the lungs and is the air that has undergone gaseous exchange with the blood. Alveolar air can also be thought of as the blood headspace [54].

Exhaled air consists of a misture of alveolar air diluted with room air retained in the dead space of the respiratory tract (mouth, nose, trachea, and bronchi). When sampling the final exhaled air, the first flow of air is discarded and only the final part of the exhalation is collected. The alveolar air is generally about two thirds of the total volume of exhaled air [10].

Consequently, exhaled air cannot be considered as a homogeneous medium, but as a mixture of air coming from different parts of the lungs, which differ in ventilation/perfusion ratios, diffusion capacities, accessibilities, etc. Therefore, upon expiration, a changing solvent concentration will be observed. Droz and Guillemin [12] showed that different breath types, integration of the concentration over different expired volumes and different possible ventilatory states of the subject, such as hypoventilated, hyperventilated or breathing normally before expiration, can lead to results that are very difficult to interpret and are sometimes quite far from the real blood concentration [12].

The fact that several authors use different sampling methods for exhaled air makes it difficult to compare results appropriately. There is an urgent need for standardization to obtain comparable results among the different research groups. Thus, the simultaneous determination of CO_2 in exhaled air may be used as a correction factor in the same way that creatinine is used for analysis of biomarkers in urine. This method would be possible since the presence of CO_2 and that of solvents are affected similarly by factors such as hypoventilation and hyperventilation, in addition to the dead space dilution. Droz and Guillemin [12] studied two types of exhalation in the determination of toluene and tetrachloroethylene in exhaled air, expressed in ppm adjusted for 5.5% CO_2. The results were similar despite the two different sampling methods, demonstrating that the sampled volume is not critical if a constant concentration of CO_2 is used as a correction factor [12]. Then, the use of CO_2 to adjust breath analysis results can be considered advantageous to compare different studies regarding occupational exposure evaluation.

In addition to the volume of exhaled air, the protocol for the time of sampling varies. Sampling at the end of the workshift has been the time of choice for evaluating occupational exposure since comparative studies show a higher sensitivity and better correlation with other indicators. However, since the sampling moment is critical, several authors have tried to minimize this problem by adopting sampling at the beginning and at the end of the work period [29]. Jo and Kim [17] evaluated the exposure of workers to aromatic solvents in dry cleaning laundries with ethylbenzene, benzene, toluene and xylene in exhaled air as exposure biomarkers. The concentrations determined at the beginning and the end of the workshift were not significantly different for benzene and toluene, while the concentrations of ethylbenzene and *m*-, *p*-, and *o*-xylene in exhaled air were significantly larger for samples collected after the workday than those collected before the workshift. The autors suggested that this result could be due to the amount of solvent used each day [17]. Table 2 shows the variety of techniques in relation to the sampling moment and the method used for sampling exhaled

Table 2
Strategies and sampling techniques of breath analysis in different occupational studies

Time of sampling	Type of breath	Sampling technique	References
12 h after workday	Total volume	Mylar bag	Money and Gray [14]
Not mentioned	Total volume	Tedlar bag	Raymer et al. [55]
Beginning and end of workday	Final volume	Glass tube	Ljungkvist and Nordlinder [56]
End of workday	Final volume	Tedlar bag inside of an aluminum tube	Dyne et al. [57]
Not mentioned	Total volume	Complex apparatus glass tube	Philips [58]
12 h after end of workday	Total volume	Tedlar bag	Peblani et al. [15]
End of workday	Final volume	Plastic bag	Jo and Kim [17]
Beginning and end of workday	Final volume	Glass tube	Egeghy et al. [29] Egeghy et al. [20]
End of workday	Final volume	Glass tube	Ghittori et al. [22]
Not mentioned	Final volume	Plastic bag	Moser et al. [37]

air in the determination of VOC. It illustrates that with a great variety of techiniques is more difficult the comparison of the reproducibility of analytical data.

One of the first alveolar air fraction sampling devices was described by Haldane-Priestley in 1905 [58]. It consisted of a rubber tube with a sampling syringe inserted in the wall close to the end of the tube. At the end of the exhalation, the tongue tip closes the tube hole, and the air sample is collected with a syringe from the stationary air column retained in the tube. After some adjustments, this technique had a variation coefficient of 18% for the analysis of hydrogen in exhaled air in bad absorption of carbohydrates studies.

Raymer et al. [59] developed a portable but complex spirometer capable of collecting alveolar air in 1.8 L canisters for later analysis by GC–MS. In operation, the subject who provided the breath sample inhaled clean air through a one-way valve and exhaled through a second one-way valve into a long Teflon tube. This breath was continually sampled into an evacuated canister. Clean air for inhalation was provided by filtering ambient air with carbon respirator cartridges. The exhaled breath was continually sampled during inhalation. By definition, the air exhaled into the tube was predominately alveolar because it was sampled mostly at the end of the exhalation. The tube was refilled with air from the next exhalation before all the air from the previous one was withdrawn [59].

Dyne et al. [57] developed a breath sampling device for capturing a portion of end-tidal air, which was transferred into an automated thermal desorption sample tube that was analysed by GC–MS. The breath sampler consisted of an aluminum outer casing narrowed at one end to form a mouth piece and, at the other end, to form an adapter that accepted Perkin Elmer automated thermal desorption tubes-ATD 400. Inside the sampler casing was a collapsible sampling bag that was attached to a movable brass ring that was guided down the length of the casing by two open channels. At the end were two non-return valves, one of which was also attached to the brass ring. The worker breathed through the sampler until the final portion of breath had been exhaled and was trapped within the sampler. The sample volume captured was 85 mL. An absorption tube was then attached to the end of the sampler, and the captured breath was forced out of the sampling bag by moving the brass ring and the non-return valve down the casing and was transferred to the absorption tube. The absorption tube was then sealed and stored until analysis by GC–MS. The authors obtained a variation coefficient between 5 and 15% for the analysis of several VOC for volunteers exposed to 10 different solvents [57].

Philips [58] developed a complex device, the “Breath Collecting Apparatus” (BCA). It was comprised of a portable microprocessor-controlled device that collected alveolar breath in an adsorbent tube. The duration and flow rate for breath collection were controlled by settings on the front panel. The subject wore a nose clip and breathed in and out through a disposable mouthpiece containing inlet and outlet flap valves. The breath sample was principally from alveolar breath. This apparatus was able to trap and concentrate the VOC contained in the alveolar breath, while allowing the nitrogen, oxygen and carbon dioxide in the breath to escape. The sample was withdrawn from a sampling port through a trap containing adsorbents such as resins or activated carbon that trapped the VOC, while most other breath compounds passed through unhindered. The styrene coefficient of variation was 11.7%. According to the author, this device had the following advantages: comfort for the individual who remained seated; an alveolar sample free of contaminants; no air vapor condensation; and viable field sampling [58].

Egeghy et al. [29] used a self-collecting breath sampling method for monitoring benzene exposure among automobile mechanics. Subjects were provided a self-collecting breath sampler and had only the written instructions for guidance. The kit used for self-measurement of solvent in breath consisted of two 75 mL glass bulbs sealed with threaded, plastic caps containing PTFE-lined septa. The samples were collected at midday and transported to the laboratory. Immediately before analysis, breath samples were transferred from the bulbs to sorbent tubes. After 24 h, the sorbent tube was removed and sealed prior to analysis. All samples were desorbed with a Perkin-Elmer automatic thermal desorption system-ATD 400 to a trap, then analyzed by GC-FID [29].

Lord et al. [60] investigated extraction with a membrane having an adsorptive interface (MESI), which they considered to be a simple and effective alternative means of performing breath analysis. The silicone membrane used for MESI breath sampling was similar in nature to that of the nonpolar lipid bilayer cell membrane of the alveoli across which many compounds must travel to be expired. MESI was developed to allow rapid routine analysis and a long-term continuous monitoring of VOC in various environmental matrices. The advantages mentioned are that the MESI unit acted as an injector; thus, it minimized analyte loss by interfacing the membrane extraction module directly to a capillary gas chromatograph. The system included a membrane module, including a flat sheet membrane to extract the analytes from the sample. The sample was exposed to one side of the membrane, and a gas flowed along the other side and transported the extracted analyte molecules into a cooled sorbent trap. The analyte was desorbed from the sorbent trap by heating and transferred for CG analysis using SPME. The results showed that MESI was a fast and quantifiable means to determine breath components [60].

All things considered, most methods have two common limitations: (1) the surface of the plastic, glass and metal containers may be adsorbants, and the losses may be significant, especially when low concentrations are to be determined; (2) the sample must be transferred to an analytical instrument, generally by using syringes. Despite its simplicity and low cost, the use of syringes leads to injection repeatability errors and to possible problems with leakage. Sampling, storage, transport and transference of the final air sample to the analysis system are critical elements for the successful analysis of exhaled air. Losses due to permeation, leaking, condensation and adsorption have been reported [60]. Many of these problems may be solved by direct analysis methods such as infrared spectroscopy and mass spectrometry [11,38]. Furthermore, the use of portable equipment enables field work and minimizes sample processing and laboratory transport losses [23,35,61]. However, the size, cost and complexity associated with these instruments make their use dif-

ficult in routine biological monitoring of workers and, therefore, makes these techniques less attractive.

A commercially available device – the Bio-VOC® sampler – has been used for research into the clinical diagnostic potential of breath. It is considered simple and affordable for occupational monitoring. The sampler captures the final portion of an exhalation, the end-expired air. This sample is then transferred into a stainless steel tube packed with an adsorbent material. Any solvents present in breath are trapped in the tube, which is sent to the laboratory for analysis. Poli et al. [41] studied a new method for breath analysis of selected VOC using the Bio-VOC® sampler and SPME. They applied it to the study of patients with primary small or non-small cell lung cancer and patients who suffer from chronic obstructive pulmonary disease [41]. The Bio-VOC® sampler was used for biological monitoring of the exposure of midwives to nitrous oxide [38].

Among breath sampling devices, some patented devices demonstrate the potential of this analysis. The patent literature also makes several references to exhaled air devices, mainly for clinical use and the detection of biomarkers of metabolic disorders. Recently presented, Patent WO No. 2005,010,482 [62] describes an invention that provides a method for analyzing one or more constituents of exhaled breath. The method includes contact of a biochip with exhaled air so that the constituent interacts with the biochip and becomes reversibly immobilized. The breath constituent is desorbed from the biochip into a mass spectrometer for identification and quantification.

7. The use of the SPME technique in the analysis of exhaled air

In recent years, there has been increased interest in the analysis of VOC in exhaled breath in clinical medicine and occupational toxicology, and over 200 compounds have been detected in human breath [63]. However, the concentration of these compounds in human exhalation is extremely low and frequently not detectable, as demonstrated by Philips et al. for lung cancer markers [43]. The low concentrations of compounds in exhaled breath make the use of a pre-concentration technique prior to analysis necessary. Pre-concentration onto a solid sorbent followed by thermal desorption is the most frequently indicated method for the analysis of exhaled air samples.

Solid-phase microextraction has been demonstrated to have a great potential in the analysis of VOC in exhaled air and has been applied to the analysis of chemical substances present in human expiration in the nanomolar range [5,64]. The SPME technique is based on the establishment of equilibrium between the analyte and a fused silica fiber coated with a stationary phase, which can be a liquid polymer, a solid sorbent or a combination of both. The analyte is then desorbed from the fiber into the injector of a chromatography system. This technique is extremely attractive since it combines analyte sampling and pre-concentration in a single process and allows direct desorption to a chromatographic system. The SPME presents low analysis cost, simplicity of operation, fiber reuse, portability, easy operation and automation, minimal sample loss and contamination during transport and storage and a large variety of phases applicable to different compounds.

The application of SPME to the analysis of exhaled air was first reported by Pawliszyn and Grote in 1997 [5]. The objective of their work was the validation of a method for determining the main endogenous compounds present in human exhalation, namely, ethanol, acetone and isoprene. The authors evaluated three fibers (85 μm polyacrylate-coated fiber; 65 μm polydimethylsiloxane/divinylbenzene-coated fiber; and 65 μm carbowax/divinylbenzene-coated fiber) with regard to sensitivity, linear range, precision and detection limits. The precision range was from 1.7 to 12.8%, although the fiber PDMS/DVB showed the best values. In this study, the SPME fiber was inserted directly into the mouth of a subject. A Teflon tube was adapted to the SPME device to protect and prevent contact with the fiber, as shown in Fig. 1.

Collection of exhaled air using SPME may be applied either passively or actively. Passive sampling requires the collection of breath in a plastic bag or some other kind of sample container for extraction at a later time. In active sampling, the individual expels breath directly onto the fiber [64]. The SPME fiber can be directly exposed in the mouth of a subject through an SPME device adapted with a Teflon tube with a small opening to the coated fiber (Fig. 1).

The first step in developing an SPME method is to determine the time necessary for the analyte to reach equilibrium with the matrix and the fiber [52]. Thus, when the exhaled air sampler is the fiber itself since it is directly exposed in the mouth of the individual, a smaller equilibration time is required. Grote and Pawliszyn [5] demonstrated that the appropriate fibers for a rather short sampling time were PDMS, PDMS/DVB and Carbowax/DVB since they reached equilibrium quickly. The results of this work showed that SPME afforded an effective method for the analysis of the compounds in exhaled air with sufficient sensitivity. However, it indicated the need for further research on the use of SPME and the use of new fibers that provide an increase

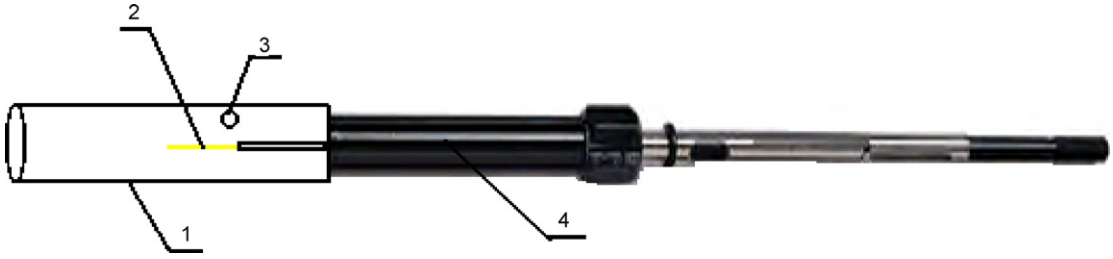

Fig. 1. Adaptation of SPME device for breath sampling (1) teflon tube, (2) exposed fiber, (3) hole, and (4) SPME device [5].

in sensitivity and selectivity for other substances of interest in human exhalation [5].

In the quest for a more sensitive analytical quantification of trace VOC in exhaled air, Giardina and Olesik [64] used low temperature glassy carbon macrofibers (LTGC) for solid-phase microextraction and analysis of 2-methylpentane, styrene, propylbenzene, decane and undecane in exhaled air. This macrofiber measured four centimeters, in contrast to conventional fibers that measure 1 cm. In this study, human exhalation was simulated, and GC–MS analyses were performed. The results showed that these fibers extracted significantly larger quantities of compounds than PDMS/DVB and had lower detection limits than those of conventional fibers [64].

There are few studies in which SPME was used in the biological monitoring of toxic chemicals. Prado et al. [65] used SPME to determine tetrachloroethylene in end-exhaled air of exposed workers. Exhaled air samples were obtained by having the subject take two or three deep breaths and then inhaling and holding the breath for 10–15 s before exhaling into the glass tube with the valves in the open position. At the end of one exhalation, the two valves were closed, trapping an aliquot of end-expired breath in the tube. The SPME fiber was introduced into the glass tube and exposed for 1 min. After extraction, the fiber was drawn into the needle, withdrawn from the tube and injected into the GC–MS [65].

Guidotti et al. [27] used SPME and GC–MS to determinate chlorinated solvents in exhaled air, urine and blood of subjects exposed in the workplace. Exhaled air samples were obtained by having the workers exhale into the container (plastic bag), the fiber was exposed to the sample for 10 min. This method allowed monitoring of subjects exposed to low concentrations of chlorinated solvents [27].

More recent work on SPME for the analysis of exhaled air for clinical purposes used the plastic bag sampling method (“Tedlar bag”) for later extraction with SPME fibers. Yu et al. [66] analyzed alkanes and aromatic hydrocarbons in exhaled air of lung cancer patients. They used PDMS fiber and tested the factors that influence the extraction of analytes such as extraction time, temperature and relative humidity. The results demonstrated the potential of SPME-GC for screening lung cancer markers [66]. Deng et al. [39] developed a new method using GC–MS and SPME with on-fiber derivatization to determinate acetone in human breath. The results showed that this method was sensitive for determination of low concentration of breath acetone and can be used as a supplemental tool for diagnosis of diabetes [39].

8. Conclusions

Breath analysis is a potential biomarker for occupational and environmental exposure since its major advantage is that it involves a noninvasive procedure. However, more research is necessary for it to be used routinely. It is especially important to establish relationships between exposures (dose) and level of substances in the various breath samples. Several types of expired air have been used in breath analysis, but it has been argued that alveolar air (end exhaled air) is the easiest sample to collect and probably the most reproducible.

Furthermore, depending on the type of breath or the sampling technique used, the results can vary considerably. Therefore, the standardization of methods is important for improved interpretation of data among subjects who are or are not exposed to VOC. For this reason, many studies must be performed. In addition, the concentration of solvent in breath may be affected by many physiological and biochemical parameters, depending on the sampling strategy.

Many of the devices described are capable of trapping an alveolar air sample and are suitable for use in field studies. However, the main disadvantage with all these techniques is the problem of loss through leakage, adsorption, formation of a water film, and transfer to the analytical system.

Among many techniques for sampling breath air, SPME is applicable for the collection of breath samples with many advantages such as direct collection, simple operation, sensitivity and selectivity. Therefore, more research on the use of SPME for application to the determination of several chemical substances of environmental and occupational interest in human breath is necessary.

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