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Influences of mixed expiratory sampling parameters on exhaled volatile organic compound concentrations

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Abstract
Breath gas analysis is a promising technology for medical applications. By identifying disease-specific biomarkers in the breath of patients, a non-invasive and easy method for early diagnosis or therapy monitoring can be developed. In order to achieve this goal, one essential prerequisite is the reproducibility of the method applied, i.e. the quantification of exhaled volatile organic compounds (VOCs). The variability of breath gas VOC measurements can be affected by many factors. In this respect, sampling-specific parameters like flow rate and volume of exhalation, exhalation with or without breath holding, exhalation in single or multiple breathing and volume of air inhaled before breath gas exhalation can play a vital role. These factors affecting the measurements must be controlled by optimizing the sampling procedure. For such an optimization, it is important to know how exactly the different parameters affect the exhaled VOC concentrations. Therefore, a study has been undertaken in order to identify some effects of different breath sampling-specific parameters on the exhaled VOC profile using the mixed expired breath sampling technique. It was found that parameters such as filling the sampling bag with high or low flow rate of exhalation, with multiple or single exhalations, in different volumes of exhalation, with breath holding and under different surrounding air conditions significantly affect the concentrations of the exhaled VOCs. Therefore, the specific results of this work should be taken into account before planning new breath gas studies or developing new breath gas collection systems in order to minimize the number of artefacts affecting the concentration of exhaled VOCs.

Introduction

Breath gas analysis has attracted more and more interest during the last years since this method enables the non-invasive monitoring of biochemical processes in the body which can be affected by the health status of a person. Therefore, many research groups have demonstrated the relationship between breath gas volatile organic compounds (VOCs) and various diseases such as unstable angina [1], breast cancer [2–4], diabetes [5–8], numerous lung diseases such as lung cancer [9–19], cystic fibrosis [20–22], COPD [23–28], asthma [29–33], pulmonary tuberculosis [34], bronchiectasis [35], pneumonia [36, 37], etc.

Much research effort has been put especially into early diagnosis of lung cancer. The list of potential lung cancer biomarkers presented in these studies is quite long but unfortunately the VOCs deemed characteristics for this disease differ from one study to another [9–19]. The reasons for that can be diverse.

One reason might be the different breath sampling methods being used in all these studies. One of the first and most frequently employed sampling methods being used in

breath analysis research is the mixed expired breath sampling technique [9, 10, 12, 19, 38–43], where a volunteer has to breathe the whole breath volume into a sample bag or container. But mixed expired air consists of dead space, transition phase and alveolar phase. The anatomical dead space and transition phase represent the first part, whereas the alveolar air the last part of the exhaled air. Only in the alveolar air are the VOC concentrations in equilibrium with the VOC concentrations emitted by the lung alveoli [41, 44]. Thus, it seems clear that the VOC concentrations in the sample strongly depend on the property of the breath sample and therefore also on the sampling technique applied. The methods from the above-mentioned lung cancer studies varied from collection of mixed expired breath gas [9, 10, 12, 19], of alveolar air as the last portion of single slow vital capacity [11], of alveolar air after breath holding for about 5 s [13], to the collection of exhaled air after inhalation of 99.99% VOC free air [14]. In addition, many other ideas have been born in order to improve the breath collection by increasing the alveolar VOC concentrations in the sample. Some examples are the CO_2-controlled sampling of alveolar breath [45], the rebreathing technique [46] or the recent development of a so-called buffered end-tidal sampler [44]. This leads to the question, which of those are the most reliable and reproducible methods in their ability to give the least intra-individual variability in the measured VOC concentration.

Another explanation for the dissimilarities in the identified biomarker of lung cancer might be the intra-individual variability over a longer period of time. This could be specified in a recent study where the day-to-day variability in measurement of exhaled VOC concentrations from a single healthy volunteer was found to be significantly high [43]. The intra-individual variability could be a consequence of various uncontrolled sampling-related parameters such as exhalation flow rate, volume of exhalation, breath holding, etc.

Various studies have demonstrated possible advantages when applying specific sampling techniques like breath holding [47], higher exhaled volumes and lower exhalation flow rate [48–52], single exhalation [49], etc which might lead to an increase in endogenous VOC concentrations in the breath samples. The major disadvantage of these studies is that they have been concentrated only on some well-known VOCs like isoprene, acetone, methanol, ethanol and acetonitrile, which makes it difficult to determine the effect on overall spectra.

The application of various breath sampling procedures in clinical studies with sick patients should be easy to perform and efficient. Most of the above-mentioned techniques may be performed only by healthy volunteers and not by lung disease patients. Thus, the necessity of reliable and easy methods for breath sampling is obvious. For such an optimization, it is important to know how exactly the different sampling parameters affect the exhaled VOCs.

Therefore, a systematic pilot study has been conducted to investigate the effects of various breath sampling parameters during mixed expired air collections on the overall VOC spectra measured between m/z 20 and m/z 200 by using proton transfer reaction-mass spectrometry (PTR-MS). Mixed expired breath gas sampling was chosen as the basic starting point of this evaluation since it was one of the first and most frequently employed sampling methods in the broad field of breath gas research, certainly due to its easily manageable and cost-efficient applicability.

Material and methods

Sampling bag preparation

Breath gas samples were collected in 3 L FEP (Teflon) bags (SKC, Pennsylvania, USA) [44, 53, 54]. In order to ensure the minimum concentration of contaminants in the reused bags, the bags were filled with synthetic air (99.99% purity) and measured to control the concentration levels. Then they were refilled with nitrogen (99.5%) and stored at 100 °C for 30 min to remove the adsorbed contaminants at the bag surface. After this, the nitrogen was flushed out. This procedure was repeated twice. At the end of this cleaning procedure, the bags were immediately filled again with synthetic air and remeasured to ensure that the VOC contaminant level is at a minimum. The VOC counts after the cleaning procedure corresponded to a ∼90% reduction compared to VOC counts before cleaning. In the case of an unusual rise in any signal after the cleaning procedure, the bag in question was discarded. In this way, the background contaminants in all the sampling bags were kept as low as possible.

Breath gas sampling

Mixed expired breath gas sampling was used as the basis for all further evaluations and as described elsewhere [9, 39, 43]. The volunteers were instructed to breathe out normally through a mouth piece up to the complete filling of the bag. The mouth piece used was relatively short (5 cm) in order to minimize condensation in this part since the mouth piece has not been heated. When volunteers had to collect more than one sample within the same experiment, they had to wait 5 min between consecutive breath collections in order to avoid stress leading to sampling artefacts. After the collection and before the directly scheduled measurement of the breath samples, the bags were stored in an oven at 40 °C for at least 30 min to evaporate all condensed water inside the bag. Based on this general method, the influence of different breath sampling parameters on the exhaled VOC profile was investigated. The specific experiments are described below.

Temperature and humidity of surrounding air. In order to study the influence of the surrounding air temperature and humidity on the exhaled VOC profile, 11 volunteers were exposed for 5 min prior to the breath collection under two different surrounding conditions (3 °C, 47% RH and 27 °C, 19% RH). The cold environment corresponded to open air during winter, while the warm environment was achieved by controlled heating of a closed room. Both experiments, the sample collection in warm and cold air, were performed directly one after another. The samples of the surrounding air were also collected simultaneously to identify any interference.

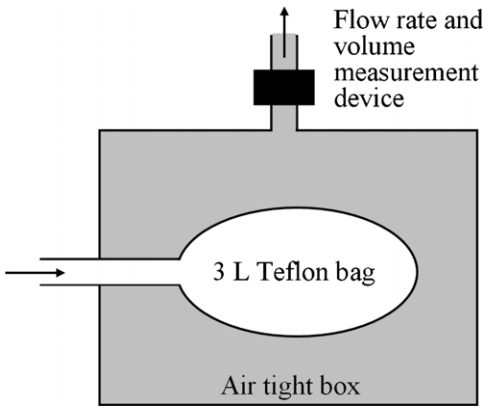

Figure 1. Setting for measuring the breath volume and flow rate.

Exhalation with breath holding. In the next experiment the effects of breath holding prior to the breath collection on the exhaled VOC profile were studied. Eleven volunteers were asked to hold the breath for 40 s before exhaling into a collection bag. For comparison, a bag was also collected from the same volunteers 5 min later without holding the breath.

Exhalation flow rate. The exhalation flow rate was measured with an indirect technique using an airtight box with one inlet and one outlet opening (figure 1). A sampling bag was fixed to the inlet hole inside the box and the outlet hole of the box was connected to a real-time flow and volume measuring device (Analyt-MTC GmbH, Müllheim, Germany). By blowing through the inlet hole into the bag, an equal amount of air inside the box was displaced simultaneously, streaming out through the outlet hole, which was then measured by the flow and volume measuring device.

This breathing experiment was carried out with two different breathing flow rate ranges (lower range: 2.5–3.5 L min^{-1}; higher range: 5.5–6.5 L min^{-1}) for a fixed exhaled volume of 2.5 L. While breathing into the bag the volunteers were able to see the display of the flow rate instrument and therefore were able to control themselves keeping the stated range. The breath samples from ten volunteers were studied for this parameter.

Volume of exhalation. To analyse the influence of the exhaled breath volume on the VOC concentrations in the sample, ten volunteers were asked to give breath samples with different volumes. Altogether 12 samples were collected per person with an end volume ranging from 50 mL to 3 L. The breath volume was measured by the earlier-mentioned measurement device (figure 1). For volumes higher than 500 mL, the volunteers were instructed to keep the exhalation flow rate constantly in the range of 2.5–3.5 L min^{-1}.

Multiple exhalations. The next experiment should evaluate whether multiple exhalations have an effect on the exhaled VOC profile. For this purpose 11 volunteers were asked to fill a bag with ten small exhalations until the bag (3 L) was filled. Prior to each exhalation, the volunteers were asked to inhale through the nose. In addition, the volunteers filled a fresh bag with a single exhalation to allow the direct comparison between the two sampling methods.

Volume of inhalation. In this experiment the influence of the inhaled volume prior to an exhalation on the exhaled VOC profile has been investigated. Eleven volunteers inhaled a known volume of room air. This was managed by using a bottle with an open bottom which was immersed in water. When the bottle was plunged into the water (by the experimenter), the volunteer directly breathed in the air coming out of the bottle neck. Bottles of two different volumes (1.5 and 3 L) were applied. The sampled exhaled volume was kept constant to 1 L by using a sampling bag of 1 L. Before starting this experiment, the volunteers were asked to empty their lungs as much as possible.

Measurement of samples with PTR-MS

The collected samples were measured by a standard PTR-MS (Ionicon, Innsbruck, Austria) as already described elsewhere [55, 56]. During the measurements, the drift tube of the PTR-MS was operated at a pressure of around 2.2 mbar and a drift voltage of 590 V. The inlet tube system (Silicosteel) and drift tube were heated and kept constant at 40 °C to avoid condensation of water from the humid gas samples. The sample flow rate was kept at 100 mL min^{-1}. The filled sample bags were heated to about 40 °C and connected at the inlet of the PTR-MS via a heated 1/8″ Teflon tube to avoid any condensation. Each sample was scanned from $m/z = 20$ to $m/z = 200$ with a dwell time of 0.5 s per m/z in seven cycles and averaged to increase the statistical accuracy [57, 58]. The concentrations presented here were calculated based on normalized count rates as described in [56] using the typical reaction rate constant for proton transfer of $k = 2 \times 10^{-9}$ cm^3 s^{-1} [59].

The ion intensity that was received is the sum of all ion concentrations with the same m/z ratio at unit mass resolution. In some cases, an identification of molecules of the same mass, which is often a problem, could be achieved with the help of the knowledge of the proton affinity and isotopic contribution of that compound. Thus, in the following work only the m/z values are presented with tentative assignments of the compounds in parenthesis.

Statistical evaluation

As the data were generally not normally distributed, analysis of the data was performed by applying a two-sided non-parametric Mann–Whitney *U*-test (*U*-test) [60] with a level of significance of 5%. Thus, the masses with z-values higher than 1.96 or lower than −1.96 were detected to be significantly different.

Results

Temperature and humidity of the environmental air

In the first experiment, the influence of the surrounding temperature and humidity on the exhaled VOC profile was

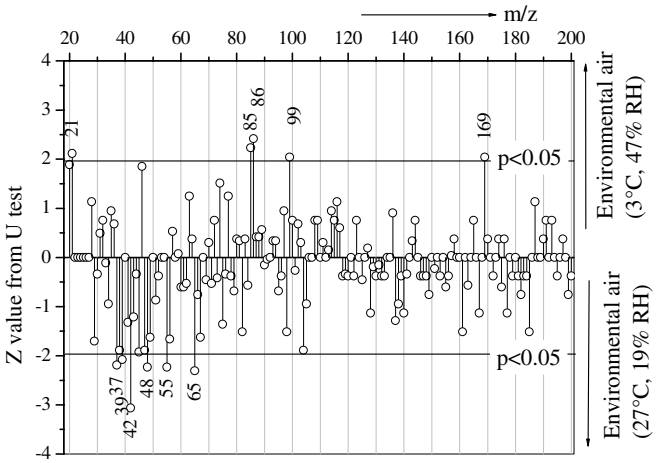

Figure 2. *U*-test between the concentrations of exhaled VOCs sampled after a 5 min stay under two different surrounding air conditions ($n = 10$ volunteers).

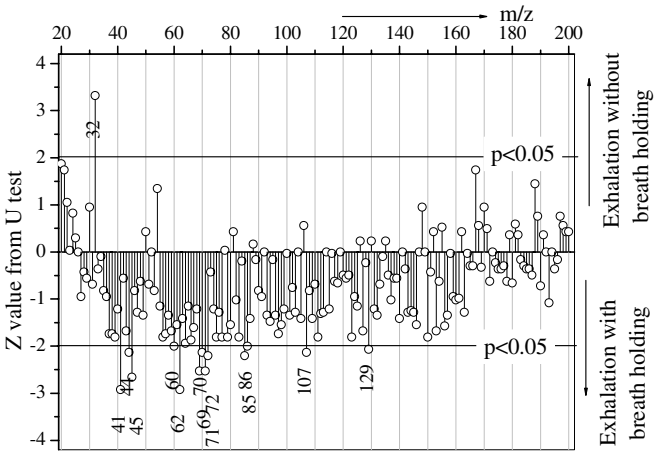

Figure 3. *U*-test between the concentrations of exhaled VOCs for exhalation with (for 40 s) and without breath holding ($n = 11$ volunteers).

evaluated. Figure 2 shows the results of the *U*-test between exhaled VOC concentrations sampled after a 5 min stay under two different air conditions (3 °C, 47% RH and 27 °C, 19% RH). It can be seen that some VOCs showed significantly higher concentrations in the samples collected under the cold air compared to the warm air condition (*U*-test, $p < 0.05$). Those VOCs were $m/z = 21$ ($H_3{}^{18}O^+$, isotope of primary ions with $m/z = 19$; dependent on the humidity of the sample), $m/z = 85$, $m/z = 86$, $m/z = 99$ and $m/z = 169$.

On the other hand, there were VOCs that revealed significantly higher concentrations in the samples collected under the warm air compared to the cold air condition (*U*-test, $p < 0.05$). Those VOCs were $m/z = 37$ ($H_2O{\cdot}H_3O^+$: water dimer, which is directly related to humidity in the breath samples), $m/z = 39$ (isotope of water dimer at $m/z = 37$), $m/z = 42$ (acetonitrile), $m/z = 48$ (isotope of ethanol at $m/z = 47$ or $NO^+{\cdot}H_2O$), $m/z = 55$ ($(H_2O)_2{\cdot}H_3O^+$ water trimer) and $m/z = 65$ ($C_2H_5OH{\cdot}H_3O^+$: cluster of ethanol). In addition, the masses $m/z = 33$ (methanol), $m/z = 43$ (propanol), $m/z = 47$ (ethanol) showed a distinct but insignificant increase in concentration (*U*-test, $p > 0.05$).

Similar changes were not observed in the comparison between the cold and warm air itself, indicating that the observed changes are due to the breath gas volatiles.

Exhalation with breath holding. Different people may behave differently when they are asked to provide breath samples. For this reason, an experiment has been performed to see whether breath holding prior to the exhalation has an influence on the VOC profile of the sample. Figure 3 represents the results of the comparison between VOC concentrations in the samples from tests with (for 40 s) and without prior breath holding. It is apparent that almost all VOCs showed higher concentrations in the breath samples collected with prior breath holding. The VOCs whose increase was significant (*U*-test, $p < 0.05$) are $m/z = 41$ (fragment of isoprene, 88.7% of $m/z = 69$), $m/z = 44$ (isotope of 1- and 2-propanol from $m/z = 43$), $m/z = 45$ (protonated acetaldehyde or carbon dioxide, whereas CO_2 has a very high concentration in the breath gas [61] so that protonated carbon dioxide may be the more likely candidate), $m/z = 60$, $m/z = 62$, $m/z = 69$ (isoprene), $m/z = 70$, $m/z = 71$, $m/z = 72$, $m/z = 85$, $m/z = 86$, $m/z = 107$ (ethylbenzene or *p*-xylene) and $m/z = 129$.

On the other hand, only one VOC revealed significantly higher concentrations (*U*-test, $p < 0.05$) in the breath sampled without breath holding, i.e. $m/z = 32$ (O_2^+: O_2^+ is produced by a backflow of the sample air from the drift tube into the intermediate ion source region of the PTR-MS [61]).

Exhalation flow rate. Depending on the individual physical capacity, the exhalation flow rate could vary throughout breath gas sampling. Therefore, an experiment had been set up to determine the effects of fast (5.5–6.5 L min^{-1}) and slow (2.5–3.5 L min^{-1}) exhalation on the sampled VOC concentrations. Figure 4 demonstrates that there was no VOC showing significantly higher (*U*-test, $p < 0.05$) concentrations in the samples collected with high compared to those collected with low flow rates. On the other hand, the concentration of some VOCs were significantly elevated (*U*-test, $p < 0.05$) in samples collected with low flow rates such as $m/z = 41$ (fragment of isoprene, 88.7% of $m/z = 69$), $m/z = 49$ (methanethiol), $m/z = 69$ (isoprene) and $m/z = 70$ (isotope of isoprene, 5.9% of $m/z = 69$).

Volume of exhalation. The next experiment should clarify whether the exhalation volume influences the concentrations of the VOCs collected in the breath sample. As shown in figure 5, the VOC concentrations indeed depended on the exhaled volume. Furthermore, it turned out that four characteristic profiles of this dependence between the exhalation volume and the VOC concentrations were typical. Examples of those are presented in figure 5.

The breath gas concentrations of VOCs like $m/z = 32$ (O_2^+) and $m/z = 47$ (ethanol/formic acid/thioformaldehyde) decreased with rising exhalation volume. While the

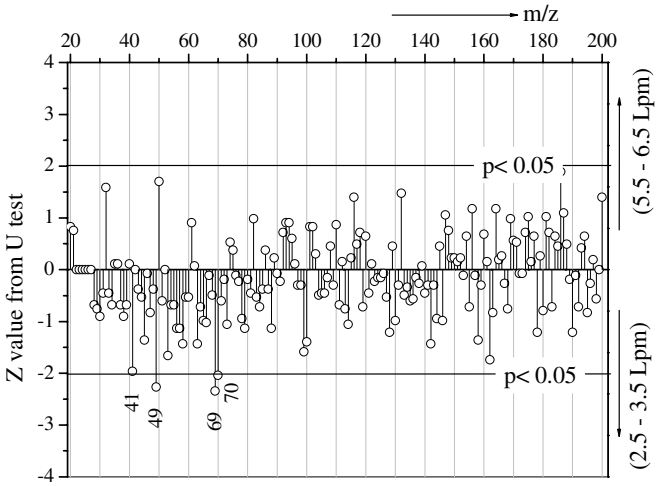

Figure 4. *U*-test between the concentrations of exhaled VOCs sampled with high (5.5–6.5 L min^{-1}) and low (2.5–3.5 L min^{-1}) breathing flow rates ($n = 10$ volunteers).

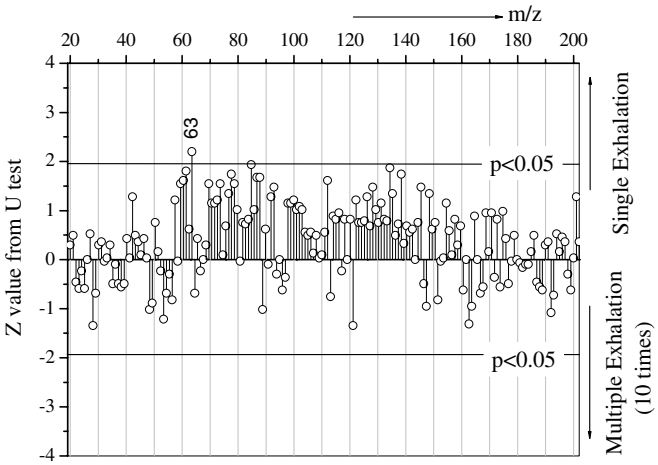

Figure 6. *U*-test between the concentrations of exhaled VOCs sampled in a 3 L bag with single and multiple (ten steps) exhalation ($n = 11$ volunteers).

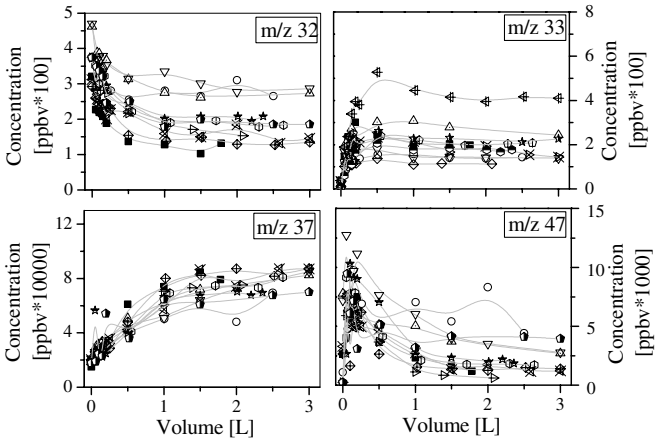

Figure 5. Exhaled VOC concentrations as a function of exhaled volume ($n = 10$ volunteers).

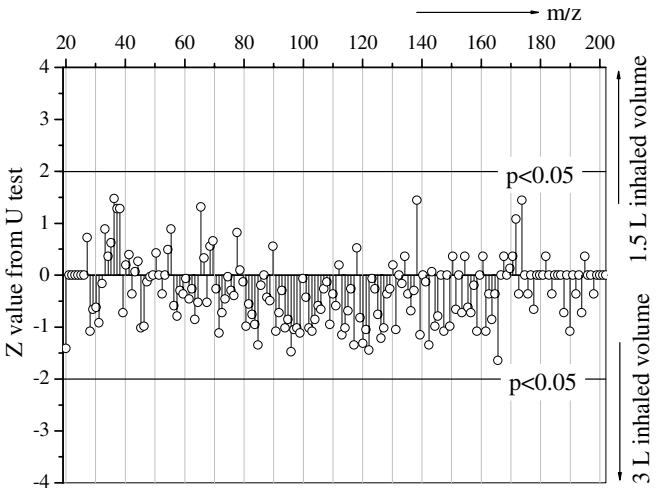

Figure 7. *U*-test between the concentrations of exhaled VOCs sampled after inhaling different volumes ($n = 11$ volunteers).

concentration of $m/z = 32$ only dropped within 0–1 L and then remained constant, other VOCs did not show such a threshold (e.g. $m/z = 47$). On the other hand, the concentrations of VOCs like $m/z = 33$ (methanol) and $m/z = 37$ (water dimer) increased with rising exhalation volume. The threshold for exhaled volume above which no relevant changes of VOC concentrations were observed lay around 1 L.

The other VOCs which have similar dependences to that of $m/z = 33$ are $m/z = 59$ (acetone/propanal), $m/z = 69$ (isoprene), $m/z = 79$ (benzene/dimethyl sulfoxide) and $m/z = 45$ (CO_2H^+) (not shown here).

Multiple exhalations. Volunteers/patients with lung problems may have difficulties while filling up a sample bag. Therefore the differences performing single or multiple exhalations regarding the collected VOC profiles have been examined. For this purpose, 11 volunteers filled a 3 L bag both with a single exhalation and in ten steps exhaling smaller volumes. It was found that filling the bag with a single exhalation produced generally higher VOC concentrations in comparison to the multiple exhalation method (figure 6). But the difference was significant (*U*-test, $p < 0.05$) only for $m/z = 63$ (water cluster of acetaldehyde, $C_2H_4O{\cdot}H_3O^+$ or water cluster of CO_2H^+, $CO_2H^+{\cdot}H_2O$).

Volume of inhalation. As already mentioned above, different volunteers may behave differently, also in terms of the inhaled volume directly prior to the sample collection. Hence, to evaluate the effect of this parameter, volunteers inhaled two different fixed amounts of air (3 and 1.5 L) and subsequently filled a 1 L bag for sample collection. It can be seen in figure 7 that most of the VOCs revealed higher concentrations in those samples being collected after the inhalation of the higher volume (3 L). However, the differences are not significant (*U*-test, $p > 0.05$).

Discussions

Temperature and humidity of the environmental air

Under different weather conditions in winter, summer and spring, the temperature and humidity levels vary strongly. In general, the room in which the sample collection takes place might be heated during winter and might be cooled during summer time. But it may also happen that the temperature and humidity in the place where breath sampling is performed, especially during summer time, is not regulated. The experimental findings at different temperatures and humidity level show that changes in the conditions of the environmental air can influence the breath gas sample measurement. For this reason, it is necessary to regulate the surrounding air and humidity to fixed conditions in order to decrease the variability in the measurement of the breath gas.

Jones *et al* [52] and Hengst *et al* [30] have shown that the alcohols in the breath gas can be influenced by many parameters like changes in the temperature of the respiratory tract after alcohol consumption or changes in the temperature and humidity of the environmental air. The alcohol content of the mucous layer has the greatest influence on the alcohol content of the breath gas. If the mucus is warmed (with inhaled warm surrounding air), so that the alcohol preferentially enters the gas phase, the solubility of alcohol in the mucus decreases resulting in a higher gas phase concentration [62]. Over a small range of temperatures close to the body temperature (35–40 °C), the relationship is approximately linear. The solubility of alcohol in water decreases approximately by 6.5% for every 1 °C increase in temperature [62].

These findings are in agreement with the results of this study where $m/z = 33$ (methanol), $m/z = 43$ (propanol) and $m/z = 47$ (ethanol) also showed increased concentrations at higher air temperature. However the results for these compounds were not significant. The reason might be that the exposure time was not long enough to significantly increase the concentrations of these compounds in exhaled gas.

On the other hand, there is also another possible explanation for lower alcohol concentrations observed in experiments performed in cold surrounding air. In this case, the breath moisture could have condensed in the cold mouthpiece so that water soluble compounds, like alcohols, may have been trapped before entering the sample bag.

The significant increase in the exhaled concentration of some VOCs ($m/z = 85, 86, 99, 169$) in cold atmosphere cannot be clarified due to the lack of information on the compound identity of these mass lines.

Exhalation with breath holding

All individuals have different exhaling styles like holding the breath or not before exhaling into the bag. This different behaviour could produce artefacts which might result in misleading measurements of certain VOC concentrations being therefore not correlated to the physiological status of a person.

The observed higher VOC concentrations in breath samples collected after breath holding might be due to the possibility of prolonged time for VOCs to diffuse between alveolar and dead space air. This phenomenon may further stimulate the exchange from blood. As a consequence the VOC concentration will rise in general. In accordance with that, Lärstad *et al* [47] have shown that the isoprene levels in exhaled breath increase with breath holding for 20 s, a consequence of the increased time available for its production.

On the other hand, the distinct difference in the exhaled concentration of the $m/z = 32$ (O_2^+) is certainly caused by the oxygen consumption during breath holding. Thus, the concentrations of $m/z = 32$ are significantly higher in samples without compared to samples with breath holding as observed in this study.

Exhalation flow rate

Depending on the individual physical capacity of the volunteers or patients tested, the exhalation flow rate may vary from collection to collection and/or from person to person. As seen in this study, many VOCs showed higher concentrations in samples collected with lower breathing flow rates compared to those collected with higher ones. This effect can be explained due to the prolonged time for the endogenously produced VOCs to diffuse from the alveoli to the periphery of the lungs allowing all in all the accumulation of higher VOC concentrations in the breath gas. Hence, the basic mechanism seems to be similar to that regarding breath holding.

In contrast to that, other research groups have found that the breath concentrations of acetone [50] and isoprene [47] were higher with higher breathing flow rates. However, in this study the respective masses $m/z = 59$ (acetone) and $m/z = 69$ (isoprene) were among those VOCs revealing higher concentrations in samples collected with lower breathing flow rates, which is in agreement with the observations from the experiments regarding the influence of breath holding.

Volume of exhalation

The results of this experiment demonstrated as expected for mixed expiratory breath sampling that the VOC concentrations are dependent on the exhaled breath volumes. The observed decrease in the breath concentration of $m/z = 32$ (oxygen) with higher exhalation volumes nicely validated the fact that the first part of the breath (dead volume) has higher oxygen concentration than the last part (alveolar air). Similarly, the increase in the concentration of $m/z = 37$ (water clusters/breath humidity) with higher exhalation volumes indicates a higher humidity concentration in the alveolar tract than in the upper respiratory region. These effects have been already described exhaustively in the literature [14, 41, 44, 45, 48–50, 62, 63].

It has also been shown that the end-expired concentration of ethanol is dependent on the exhaled volume, on the flow rate and the temperature of environmental air [48–52]. Hlastala *et al* [48] have shown for one subject that the breath alcohol concentration increases continuously as the subject exhales. However, the results obtained with ten subjects in this study clearly indicated an opposite finding, i.e. that the

concentrations of $m/z = 47$ (ethanol) are much higher in the first parts of the breath rather than in the posterior (alveolar) parts. This could be due to higher ethanol concentration in the upper respiratory tract compared to that in the alveolar air, e.g. due to mouth space contamination since no rinsing has taken place before sampling. The collection of higher and higher volumes will then lead to a dilution of the overall breath ethanol concentration in the sample.

The experiments also indicated a threshold for the expired volume of about 1–1.5 L above which no relevant changes of VOC concentrations could be observed. This finding may be helpful for the planning of further breath gas tests and how to handle the dead volume which is specified to be about 150 mL [14, 48]. In order to get rid of the influences of the dead volume, it is necessary to discard the volume up to the saturation limit of each VOC. The results of this study suggest considering the first 1–1.5 L of breath gas as a volume which has to be discarded meaning more or less a step towards alveolar sampling.

Multiple exhalations

This experiment revealed that breath samples collected with multiple exhalations generally contain lower VOC concentrations than samples collected with a single exhalation. The reasons for that might be due to the influence of the anatomical dead space volume. In multiple exhalations, a certain amount of dead space air is exhaled into the sample each time. This will dilute the breath and therefore decrease the VOC concentration in the sample bags. Hence, it is advisable to collect the breath samples with single exhalations rather than with multiple exhalations, although the differences in the current work were not significantly high.

Volume of inhalation

Higher inhaled volumes may dilute the alveolar air leading to a decrease of VOC concentrations in the exhaled air. In contrast, the results of this study showed that higher inhaled volumes seem to lead to slightly elevated VOC concentrations. However, the results were not significant.

Conclusion

This work shows that various sampling-specific parameters may influence the outcome of a breath gas test when mixed expiratory sampling is employed. The parameters such as breath holding, low exhalation flow rates, higher exhaled volumes and single exhalation have been shown to increase the VOC concentration in the mixed expired breath gas sampling. Hence, these techniques could be used according to the specific interest in certain VOCs to increase or decrease their concentration in the breath gas samples in comparison to their concentration in the surrounding air. This is an important possibility to minimize the variability of the concentrations of exhaled VOCs. To minimize the sampling-induced variations in the breath gas VOCs, the consideration of all these sampling-specific factors seems to be inevitable. Hence, the specific results of this study can be used as input for the planning of further breath gas studies. Altogether, the outcome of this study strengthens the conclusion that only the controlled use of alveolar air seems to be appropriate for measuring the endogenously produced VOCs and therefore seems to be a more promising way towards sound results in breath gas tests.

Acknowledgments

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