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Abstract
We have measured the acetylene concentration in the exhaled breath of 40 volunteers (31
non-smokers, nine smokers) using near-infrared cavity ring-down spectroscopy. The acetylene
levels were found to be the same as in ambient air for non-smokers, whereas elevated levels
were observed for smokers. Real-time measurements with sub-second time resolution have
been applied to measure the elimination kinetics of acetylene in breath after exposure to
tobacco smoke. Three exponential time constants can be distinguished from the data and these
can be used to define the residence times for different compartments, according to the
multi-compartment model of the human body.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Acetylene (C_2H_2) is a common hydrocarbon in the troposphere originating almost exclusively from anthropogenic sources [1]. The main sources of acetylene in the atmosphere are different combustion processes: vehicle exhausts and biomass burning. The outdoor acetylene concentration in an urban environment is normally about 1 part per billion by volume (ppbv). In a recent study, we measured indoor and outdoor acetylene concentrations in Helsinki, Finland and found highly fluctuating daytime outdoor acetylene levels [2]. The mean mixing ratio was 2 ppbv or below for both indoor and outdoor settings. In atmospheric studies, acetylene is often used as a marker for anthropogenic emissions and to trace polluted air masses. The acetylene concentration in the atmosphere correlates well with the concentrations of other volatile organic compounds (VOCs) [3] and that of carbon monoxide [4].

Acetylene has previously been detected in exhaled breath [5], but the concentration has not been quantified, to the best of our knowledge (except for one breath sample recorded by us [2]). Unless the human body acts as a sink for acetylene, one would expect to find levels that are at least as high as the concentration in ambient air. Short periods of exposure to high

levels of combustion products might give rise to an elevated acetylene concentration in the body. As one cigarette has been found to release about 150 μg of acetylene to the environment [6], it seems reasonable to assume that smokers exhibit higher concentrations of C_2H_2 in breath than non-smokers. Some bacteria have also been shown to produce small hydrocarbons like acetylene [7], and thus it is also possible that C_2H_2 is endogenously produced in the human body.

Different molecules have been suggested as biomarkers for the smoking status of an individual, using different matrices including urine, blood, hair, saliva and exhaled breath [8]. The most widely used biomarker in breath is carbon monoxide [8, 9] but other compounds, such as acetonitrile [10, 11], benzene, 1,3-butadiene and 2,5-dimethylfuran [12, 13] have also been suggested. Of these molecules, 2,5-dimethylfuran seems to be most effective since it can be observed in breath more than 24 h after smoking and can even be used to detect heavy passive smokers and occasional social smokers [13].

Real-time trace gas analysis can be used to monitor the uptake and elimination kinetics of various compounds in breath [14–16]. This enables the execution of real-time pharmacokinetic studies, which aim to establish the processes and pathways of foreign chemical species in the

body. The elimination usually follows a multi-exponential function where the two shortest time constants are on the order of several seconds and a couple of minutes, corresponding to washout from the lungs and release from the blood, respectively [14, 15]. In order to obtain enough data points to characterize the decay function at these short time scales, a real-time measurement without pre-concentration steps has to be used. Real-time analysis of trace gas species at ppbv levels in breath is challenging because the required sensitivity must be combined with a short acquisition time. To monitor a normal breathing cycle at about 10 breaths per minute, a detector response of less than one second per measurement point is required. Traditional techniques, such as gas chromatography combined with mass spectrometry (GC–MS) cannot reach such short acquisition times. However, proton-transfer reaction mass spectrometry (PTR-MS) [17], selected ion flow tube mass spectrometry (SIFT-MS) [18] and various types of sensitive laser absorption spectroscopy [19, 20] are able to reach the required acquisition times at ppbv detection levels in breath.

In this work, we present the results of a study where we have measured the acetylene concentration in the sampled breath of 40 volunteers. Additionally, we have measured the elimination kinetics of acetylene after cigarette smoking for five volunteers. The acetylene concentration was followed in real time for 1 h with short pauses in-between. The data have been fitted to a multi-exponential function that gives the residence times for acetylene related to different hypothetical compartments used to model the human body [21]. We have also measured acetylene mixing ratios in headspace samples of *Proteus mirabilis* cultures.

2. Materials and methods

2.1. Human subjects

Breath samples were collected from co-workers and students at the Laboratory of Physical Chemistry, University of Helsinki. All gave their written informed consent to participate in the study. The research was approved by the co-ordinating ethics committee of the Hospital District of Helsinki and Uusimaa. No special inclusion criteria were required and no dietary controls were imposed. For the background concentration study, a total of 40 samples were collected from volunteers aged 20–63 years: 22 men and 18 women, of whom 31 were non-smokers and nine were smokers. For the elimination kinetics measurements, five volunteers took part, aged between 25 and 34 years: four men and one woman, all of whom were light or occasional smokers.

2.2. Breath collection

For the acetylene concentration measurements, each subject provided a single exhaled full breath sample that was collected by direct breathing into an aluminum-coated sampling bag of 1300 cm^3 volume (Wagner Analysen Technik, WT 8004). Simultaneously, a second sample bag of the same type was filled with indoor air from the room the subject was occupying. All samples were taken indoors. The volunteers were required

to have stayed in the room for a minimum of 30 min before the sampling took place. The bag samples were analyzed on the same day or the following at the latest. The bags were verified to be suitable for acetylene sampling; they were found to retain the same C_2H_2 mixing ratio for up to 5 days [2]. Moreover, we did not observe adsorption/desorption phenomena in any part of the gas transfer line or the sample cell. The samples were vacuum-extracted to the sample cell via a Nafion tube (PermaPure, MD-070-72) to dehumidify them. Acetylene mixing ratios were found not to be affected by this process. The cell was kept at room temperature (22 °C) and was filled to 100 mbar pressure.

To study the real-time elimination kinetics after cigarette smoking, the volunteers were asked to breath continuously into a sampling mouthpiece. The mouthpiece consisted of a bacterial filter (Vitalograph, 28350), a two-way non-rebreathing valve (Hans Rudolph Inc, 1410 series) and a 35 cm^3 volume buffer tube from top of which the gas sample stream was transferred to the sample cell. A nose clip was used to prevent nose breathing. The gas flow rate to the cell was kept at 1000 cm^3 min^{-1} using a mass-flow controller (MKS, M100B). The Nafion tube was used and the cell pressure and temperature were the same as in the case of the bag measurements. The volunteers were required to have refrained from smoking for at least 2 h before the measurement. The subjects smoked a cigarette, exhaled and inhaled normally 3 times and then started to breathe into the mouthpiece. The same brand of cigarettes was used in every measurement. The breathing sequence was as follows: 15 min of continuous breathing into the mouthpiece, 2 min of rest, 5 min of breathing, 2 min of rest, 5 min of breathing, 5 min of rest, 5 min of breathing, 5 min of rest, 5 min of breathing, 5 min of rest and 5 min of breathing. The pauses were taken to make the measurement more convenient and agreeable for the volunteers. An audible metronome was used to help the subjects keep a steady respiration rate of 12 breaths min^{-1}, with equal durations of inspiration and expiration. To measure the fast washout process from the lungs, separate 5 min measurements were made at a rate of 20 breaths min^{-1}. For these measurements the subject blew the tobacco smoke out of the mouth after finishing the cigarette but held his breath until starting to breathe into the mouthpiece.

2.3. Cavity ring-down spectrometer

Cavity ring-down spectroscopy (CRDS) is a highly sensitive absorption technique that exploits the interaction of a laser beam with an optical cavity consisting of two or more highly reflective mirrors [19, 22]. Details on the continuous-wave CRD spectrometer used in this study are given in [2]. For the measurements made using sample bags, the spectrometer was essentially the same as in that reference, the only difference being the addition of the above-mentioned Nafion tube. For the elimination kinetics measurements, the 500 cm^3 ring-down cell was exchanged for a 40 cm^3 cell (of the same length) to enable a shorter gas exchange time. The smaller cell was also made of stainless steel but was not quartz-coated like the 500 cm^3 cell. At 1000 cm^3 min^{-1} gas
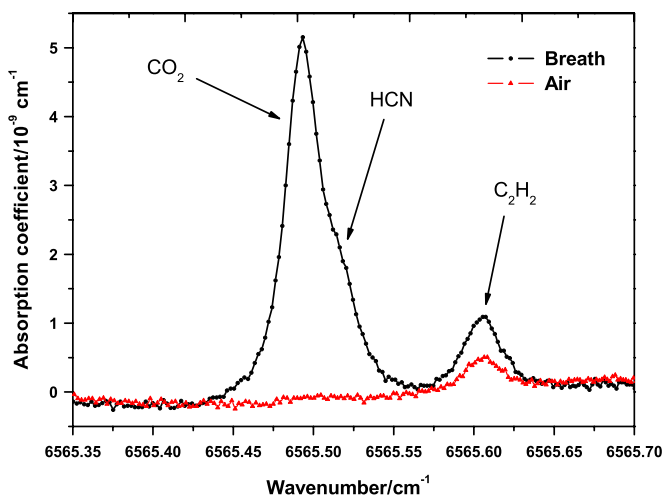
Figure 1. Typical spectrum for the acetylene concentration measurements. Black circles represent experimental data points for a breath sample and red triangles indicate simultaneously sampled room air. The acetylene mixing ratios are 2.0 ppbv and 0.8 ppbv, respectively (smoker, no 2). The baseline has not been corrected for a weak etalon effect.

flow rate and 100 mbar sample pressure, the theoretical gas exchange time inside the cavity was about 240 ms. During the process of exchanging the sample cells, the high reflectivity mirrors were contaminated and subsequently the ring-down time constant (and hence the sensitivity) dropped from about 210 μs (concentration measurements) to about 110 μs (elimination kinetics measurements).

To measure the acetylene mixing ratio from the sample bags, the laser wave number was scanned over the region 6565.35–6565.75 cm^{-1}. A typical spectrum is shown in figure 1. The wave number range includes the acetylene absorption line at 6565.620 cm^{-1} ($\sim$1523 nm), a carbon dioxide line at 6565.509 m^{-1} and a hydrogen cyanide line at 6565.532 cm^{-1}. This region was chosen as a compromise between maximal acetylene line strength and minimal spectral overlap with other species. For each measurement, four scans were co-added, each consisting of 210 points with 25 ring-down events averaged per point. The total recording time for these measurements was about 10 min/sample. The estimated detection limit for acetylene was 175 pptv (three times the standard deviation).

High time resolution was needed to measure the elimination kinetics after cigarette smoking. For this reason, the laser wave number was kept fixed (without active stabilization) at the top of the acetylene absorption line. The wave number was monitored using a wave meter (EXFO, WA-1500), which has a frequency uncertainty of $\pm$40 MHz. The full width at half maximum linewidth of the acetylene transition is about 850 MHz. Altogether 20 ring-down events were averaged for each point. The repetition rate for ring-down decays was 40 Hz. Thus, the time resolution of the experiment was about 500 ms. The estimated detection limit for these measurements was 750 pptv (three times the standard deviation).

Since we did not scan over the acetylene peak in the kinetics measurements, we cannot distinguish the contribution

of acetylene absorption from any other species that absorbs at this wavelength. In fact, the wing of the HCN absorption line that lies close to the acetylene peak introduces a measurable contribution to the net absorption. Tobacco smoke contains a lot of HCN (up to 380 μg/cigarette [23]) and we have measured levels as high as 900 ppbv in exhaled breath directly after smoking. Thus, the rate of decay of HCN in breath will cause a slightly changing baseline to our measurement and introduces a small error to the C_2H_2 concentrations. This effect is, however, a minor one; we estimate that HCN contributes at most 5% to the overall absorption measured at the top of the acetylene absorption peak.

2.4. Data analysis

The spectra from the background level study were analyzed by fitting the absorption lines to Voigt functions. All three lines (CO_2, HCN and C_2H_2) were fitted simultaneously and a sine function was included to account for a weak etalon background [2]. The acetylene peak area was then converted to a mixing ratio using the line strength of the transition (8.385 $\times$ 10^{-21} cm molecule^{-1}), which is known to an accuracy of better than 2% [24]. The line fitting was performed using the software Fityk [25]. For the elimination kinetics study, the ring-down time constant was recorded, compared to the empty cavity ring-down time, converted into absorption units and the C_2H_2 concentration was calculated using the line strength of the transition, assuming a constant linewidth at constant pressure.

The evolution of the acetylene concentration in breath as a function of time after smoking can be expected to follow a multi-exponential function. The different exponential time constants are then related to compartments in the human body, such as the blood and different types of tissue. Wallace *et al* [21] developed a linear mass-balance multi-compartment model that can be used to model the uptake and decay of compounds in the body. Because we observed only the elimination of acetylene, for our purposes, a simple multi-exponential decay function suffices:

$$C_{\text{HCCH}} = \sum A_i \exp \frac{-t}{\tau_i}, \qquad (1)$$

where C_{HCCH} is the acetylene concentration in exhaled breath, A_i is the fractional acetylene contribution of the ith compartment to the breath at equilibrium, t is the time measured from the end of exposure and τ_i is the residence time of acetylene in the ith compartment. The acetylene concentration at time t is obtained from the experimental data by finding the maximum of each exhalation cycle. These maxima were then fitted to a multi-exponential decay function using the Origin 7.5 (Originlab) software. For the 1 h measurements, statistical weighting ($w_i = 1/y_i$, where y_i is the value of the ith data point) was applied in the fitting process to account for the larger absolute error associated with high acetylene concentration points. The baseline acetylene concentration is assumed to be zero, which actually corresponds to the small acetylene level present in ambient air (about one ppbv). Fitting of the 5 min measurements, where

Table 1. Acetylene concentration measurements made using sample bags. Values are acetylene mixing ratios in breath and in air in units of ppbv.

	C_{breath}	C_{air}	$C_{breath} - C_{air}$
Smokers			
1	2.29	1.28	+1.01
2	2.00	0.82	+1.18
3	3.05	1.50	+1.55
4	3.08	1.47	+1.61
5	3.57	1.52	+2.05
6	9.24	0.84	+8.40
7	3.72	0.81	+2.91
8	5.68	1.31	+4.37
9	7.86	1.19	+6.67
Non-smokers			
1	1.39	1.35	+0.04
2	1.31	1.38	−0.07
3	1.25	1.21	+0.04
4	1.34	1.23	+0.11
5	1.22	1.17	+0.05
6	1.68	1.72	−0.04
7	1.09	1.00	+0.09
8	1.02	0.97	+0.05
9	1.01	0.94	+0.07
10	0.92	0.89	+0.03
11	0.90	0.93	−0.03
12	0.78	0.71	+0.07
13	0.78	0.74	+0.04
14	0.73	0.72	+0.01
15	1.66	1.56	+0.10
16	1.50	1.47	+0.03
17	1.50	1.46	+0.04
18	1.40	1.31	+0.09
19	0.89	0.86	+0.03
20	0.83	0.86	−0.03
21	0.92	0.82	+0.10
22	1.30	1.23	+0.07
23	1.29	1.22	+0.07
24	1.28	1.26	+0.02
25	1.24	1.17	+0.07
26	1.15	1.13	+0.02
27	1.12	1.15	−0.03
28	1.21	1.19	+0.02
29	0.93	0.84	+0.09
30	0.99	0.87	+0.12
31	2.66	2.66	+0.00

the dynamic contrast of the data was smaller, was done without weighting.

Comparisons between groups (gender, smoking) and correlations (C_2H_2 and HCN in breath, age) were performed using the Mann–Whitney U and Spearman's rank correlation tests (PASW Statistics 18, SPSS Inc.), respectively.

3. Results

3.1. Background level and bacterial measurements

The results of the acetylene concentration measurements that were made using sample bags are presented in table 1. Values are given for C_2H_2 mixing ratios in exhaled breath, ambient air of the room and the difference between these two values. It is clear that smokers exhibit significantly higher acetylene

levels in breath than non-smokers. As mentioned above, the subjects were required to have stayed in the room where the sampling took place for at least 30 min. As smoking is not allowed inside the University, there were always at least 30 min between the last cigarette and the sampling event. No additional information was available on the smoking status of the volunteers.

The distribution of the difference of breath and air concentrations for non-smokers is not exactly centered around zero (mean +40 pptv, standard deviation 47 pptv). Such small deviations are, however, on the same order of magnitude as our determined error for acetylene measurements made using sample bags [2]. They could also be caused systematically by the absorption line fitting procedure as breath contains more CO_2 and HCN than ambient air and the peaks of these compounds can slightly influence the obtained acetylene mixing ratio. The C_2H_2 concentration in the breath of a non-smoker can therefore be considered to be the same as in ambient air. The mean acetylene mixing ratio for the indoor air samples is 1.17 ppbv, which is within the variation (between 0.5 ppbv and 2.0 ppbv) measured in our laboratory air previously [2]. It is also close to the mean level of 1.05 ppbv measured for 14 indoor air samples from various locations around Helsinki [2].

In addition to smoking, exposure to other types of combustion products can cause higher levels of acetylene in exhaled breath. One of the authors of this study spent a few minutes in an indoor car parking lot, where the ambient acetylene level was about 55 ppbv. After 75 min, the concentration in his breath was still slightly higher than in the ambient laboratory air (760 pptv in breath, 580 pptv in air).

We also measured the HCN mixing ratios of the breath samples, using the absorption line at 6565.532 cm^{-1}. The mean HCN concentration was 5.3 ppbv (median 4.1 ppbv, standard deviation 3.7 ppbv), which is close to the value measured previously by us [26]. Smokers had significantly higher levels of HCN compared to non-smokers. The HCN and C_2H_2 mixing ratios did not show a statistically significant correlation (correlation coefficient 0.27) in Spearman's rank test. This might be partially due to the sample bags because the HCN mixing ratio decreases during the storage in the bag. More importantly, the (not smoking influenced) HCN baseline level as measured from a mouth exhaled sample is higher than the acetylene level and this baseline is thus reached faster for HCN than for C_2H_2.

We also wanted to investigate the possible acetylene production of *Proteus mirabilis in vitro* [7]. To this effect, we grew the bacteria and measured headspace samples from two 500 cm^3 containers. The bacteria (strain ATCC 29906) were incubated for 24 h at 37 °C on prepared Columbia Agar plates with 5% sheep blood (PB5039A, Thermofisher Scientific). The measured acetylene concentrations were the same as in a control sample container without bacteria (the ambient air level). In [7], a nutrient broth was used to grow the bacteria and the authors measured an acetylene production rate of 0.1 nl / (ml of nutrient broth) × hour. Supposing both methods (nutrient broth and agar plate) produce comparable bacterial
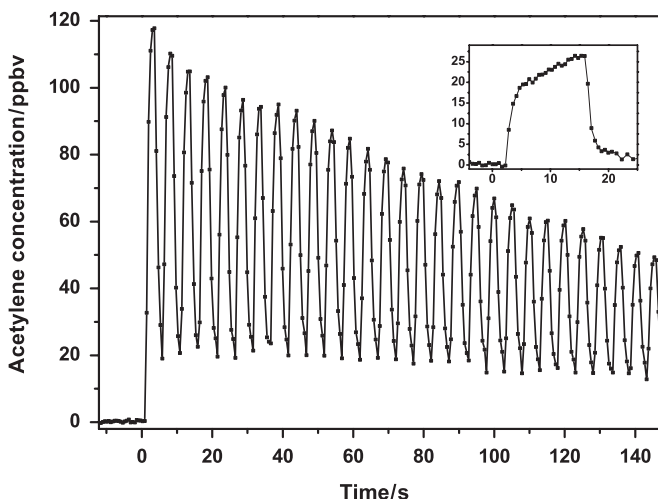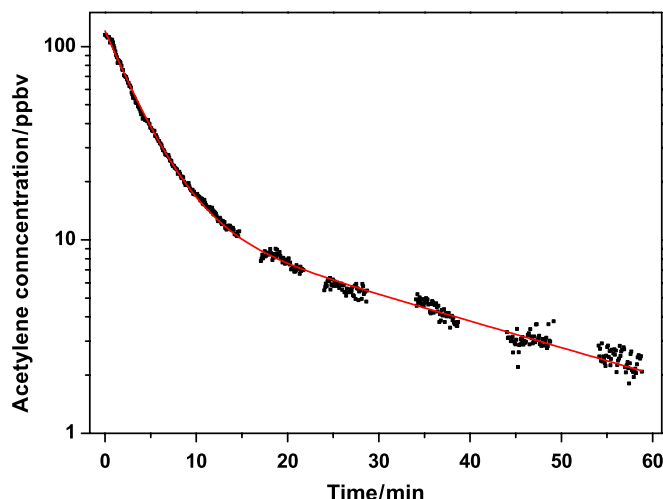
Figure 2. Real-time measurement of acetylene concentration in exhaled breath after cigarette smoking. Paced respiration rate of 12 breaths min^{-1} was used. The inset shows a longer exhalation, where the alveolar slope is visible. The units in the inset are the same as in the main graph for both axes.

numbers and both media support acetylene production equally strong, we should have observed C_2H_2 concentrations of tens of ppbv. Thus, we are unable to reproduce the result obtained in [7], although the difference in the growth media should be emphasized.

3.2. Elimination kinetics after exposure to tobacco smoke

Figure 2 shows a sample of a real-time measurement of the acetylene mixing ratio in exhaled breath after cigarette smoking. The time resolution of the experiment was high enough to reveal the evolution of a single breath cycle at a paced respiration rate of 12 breaths min^{-1}. The inset in figure 2 shows a longer exhalation, where the alveolar slope is clearly visible. During the inhalation, the C_2H_2 concentration does not go to zero; this is due to the size of the buffer tube volume compared to the gas flow rate through the spectrometer. The acetylene-rich air in the buffer tube is not completely exchanged during the inhalation cycle because the inhaled air comes from the other side of the non-rebreathing valve.

The acetylene elimination in breath was followed for 1 h after smoking one cigarette. Paced breathing at 12 exhalations per minute was used. A representative decay graph is presented in figure 3, where each individual point corresponds to a maximum of an exhalation cycle as explained in section 2.4. Altogether five 1 h measurements were made for subject no 1 and one measurement each for subjects no 2–5. The decay parameters for subject no 1 are given in table 2 and for subjects no 2–5 in table 3. The data were fitted with two exponential decay functions according to equation 1. The longer time constant likely represents tissues and the shorter one blood [21]. A systematic trend was noticed, where the first few points of every measurement cycle (when subject starts to breathe into the mouthpiece) correspond to lower acetylene concentrations than the following ones. For this reason, the first three points of every measurement cycle

Figure 3. One hour measurement of acetylene elimination kinetics after smoking, semi-logarithmic plot. Black full circles are the experimental data points (subject no 1, measurement no 1) and the red line corresponds to a nonlinear least squares fit with two exponential decay functions.

Table 2. Acetylene decay parameters after cigarette smoking, subject no 1, 1 h measurements at 12 exhalations min^{-1}. Parameters A_i and τ_i refer to equation 1. Uncertainties for parameters are standard errors of the nonlinear least squares fit. Stdev is standard deviation.

Measurement	A_2, ppbv	τ_2, s	A_3, ppbv	τ_3, s
1	107.0 ± 2.7	217.9 ± 8.3	13.5 ± 1.1	1890 ± 148
2	117.1 ± 3.0	192.5 ± 7.0	14.6 ± 1.1	1731 ± 116
3	68.7 ± 2.7	163.2 ± 9.4	15.5 ± 1.0	1454 ± 82
4	90.2 ± 2.9	157.4 ± 6.9	12.2 ± 0.9	1517 ± 99
5	76.4 ± 2.6	183.0 ± 8.6	11.5 ± 0.9	1914 ± 146
Mean	91.9	182.8	13.5	1701
Stdev	20.3	24.3	1.7	210

Table 3. Acetylene decay parameters after cigarette smoking, subjects no 2–5, 1 h measurements at 12 exhalations min^{-1}. Parameters A_i and τ_i refer to equation 1. Uncertainties for parameters are standard errors of the nonlinear least squares fit. Stdev is standard deviation.

Subject	A_2, ppbv	τ_2, s	A_3, ppbv	τ_3, s
2	261.8 ± 5.0	151.3 ± 3.9	37.3 ± 1.5	1418 ± 49
3	151.5 ± 4.0	179.0 ± 7.2	40.9 ± 1.6	1650 ± 58
4	91.0 ± 3.6	118.0 ± 6.2	19.7 ± 0.9	1452 ± 59
5	199.5 ± 4.5	157.6 ± 5.1	34.3 ± 1.7	1237 ± 45
Mean	176.0	151.5	33.1	1439
Stdev	72.4	25.3	9.3	169

were deleted from the dataset. This artifact is probably due to the subjects adjusting themselves to breathing through the mouthpiece. It is known that the use of a mouthpiece and a nose clip influences the breathing pattern and increases the tidal volume [27].

To measure the fast washout process from the lungs, we also made 5 min short measurements of the acetylene elimination process with a higher ventilation rate, 20 exhalations per minute. In these measurements, a third

Table 4. Acetylene decay parameters after cigarette smoking, subject no 1, 5 min measurements at 20 exhalations min^{-1}. Parameters A_i and τ_i refer to equation 1. The longest time constant (τ_3) is fixed to 1600 s in the fitting procedure. Uncertainties for parameters are standard errors of the nonlinear least squares fit. Stdev is standard deviation.

Measurement	A_1, ppbv	τ_1, s	A_2, ppbv	τ_2, s	A_3, ppbv	τ_3 (fixed), s
1	32.0 ± 1.7	2.1 ± 0.3	113.2 ± 0.8	119.3 ± 2.7	26.5 ± 1.0	1600
2	25.3 ± 1.7	3.9 ± 0.6	127.0 ± 0.9	121.8 ± 2.8	27.5 ± 1.1	1600
3	7.1 ± 1.6	3.0 ± 1.7	94.1 ± 1.5	162.8 ± 6.5	18.1 ± 1.9	1600
4	50.8 ± 2.5	7.0 ± 0.7	116.4 ± 3.6	186.9 ± 13.3	9.4 ± 4.7	1600
Mean	28.8	4.0	112.70	147.7	20.4	
Stdev	18.0	2.1	13.7	32.9	8.4	
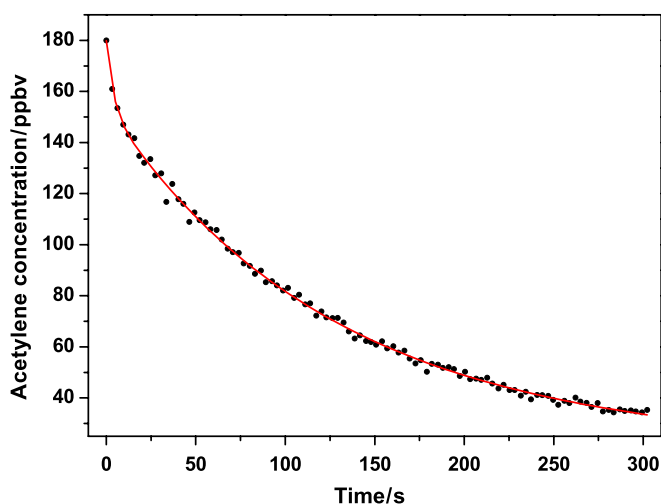
Figure 4. Five minute measurement of acetylene elimination kinetics after smoking. Black full circles are the experimental points (subject no 1, measurement no 2) and the red line corresponds to a nonlinear least squares fit with three exponential decay functions.

exponential component with a time constant in the order of few seconds was visible. Altogether four of these measurements were made with subject no 1, the decay parameters are summarized in table 4. A representative graph is presented in figure 4. No points were discarded in the analysis of these measurements. Three exponentials were fitted to these data; the time constant of the slowest one was fixed to 1600 s but the amplitude was left to vary.

4. Discussion and conclusions

The C_2H_2 concentration measurements made using sample bags demonstrate that the acetylene mixing ratios in the breath of non-smokers are the same as in ambient air. Smokers exhibit higher mixing ratios in their breath compared to ambient air and this makes it possible to distinguish active smokers from non-smokers. Within the admittedly limited population of our study the sensitivity and specificity for the detection of smokers were 100%. However, based on the elimination kinetics measurements, acetylene cannot be used to detect smokers for a very long time after the last cigarette. After 2 to 3 h, the acetylene level will return to that of ambient air. In that respect, acetylene cannot compete as a biomarker for smoking status with molecules like 2,5-dimethylfuran, which can be detected at elevated levels more than 24 h after the

last cigarette [13]. An additional complication arises because acetylene is not a truly specific marker for tobacco smoke since exposure to other types of combustion products will also result in an elevated acetylene level. The advantage of acetylene is that it does not seem to be produced endogenously in healthy subjects and thus the baseline level in breath is very low. This is in contrast to, for example, carbon monoxide, which is present at parts-per-million by volume (ppmv) levels even in the breath of non-smokers [9].

The elimination kinetics measurements after smoking demonstrate the power of highly sensitive real-time laser spectroscopy. We can even distinguish the rapid wash-out process from the lungs that happens on the time scale of seconds. In the case of exposure to tobacco, this washout is of limited meaningfulness. Looking at the results in table 4, especially measurement no 3, it is clear that there is a large intra-individual variation in the amplitude and time constant of this fastest decay process. This is most likely due to differences in how the cigarette is smoked and how deep the last puff is. If the exposure was done in a more controlled way, the washout phenomenon from the lungs would most likely be more repeatable. However, the number of data points (even at 20 breaths min^{-1}) is small for the time scale of the fast decay, resulting in a fairly inaccurate determination of this parameter. For the longer time constants, the inter- and intra-individual variation is smaller. Amplitudes of the decay are clearly dependent on the smoking style but the values of the time constants are quite reproducible. Furthermore, as is seen in table 2, for the same subject, even the amplitudes are reproducible from measurement to measurement. Comparing the 5 min and 1 h measurements for subject no 1 it seems that a faster respiration rate (20 breaths min^{-1} versus 12 breaths min^{-1}) results in a shorter time constant for the second compartment.

Breathing through the mouthpiece for extended times is not trivial and some people find it more difficult than others. Variations in breathing technique during the measurement are reflected in the retrieved instantaneous acetylene levels and cause irregularities in the decay curve. Thus, the quality of the elimination data varies significantly from subject to subject. Reassuringly, however, the fitted decay parameters are reproducible and do not seem to be affected by minor artifacts in the data.

The total averages over all measurements (12 breaths min^{-1}) for the second and third time constants (tables 2 and 3) are 169 s and 1585 s, respectively. These values compare

reasonably well with observations for other VOCs. Von Basum *et al* [14] measured mean values (three subjects) of 49 s and 1110 s for the second and third compartments, respectively, after exposure to ethane. They also report the mean value for the first compartment (washout from lungs) as 18 s but due to the low respiration rate used in that study (4 breaths min^{-1}) they had only a few data points on the time scale of the first decay process. For the elimination of benzene, 1,3-butadiene and 2,5-dimethylfuran after smoking, Gordon *et al* [12] used a two-compartment model and retrieved mean values (five subjects) between 30–50 s for the second compartment for the different compounds and about 840 s for the third. For trichloroethene, mean values (six subjects) of 210 s and 3780 s were measured for the second and third compartments, respectively [28].

It is quite clear that the largest errors in our measurements are not connected with the laser spectroscopic analysis. The bottleneck is rather the sampling of the breath. In order to control and standardize the sampling event, simultaneous recording of the tidal volume and CO_2 concentration could be used to characterize the end-tidal part of the exhalation cycle [29]. The bag samples collected in this study were full breath samples and were thus diluted with the dead-space contribution from the mouth and airways. For non-smokers, we could not find any difference in acetylene mixing ratio between the beginning of the exhalation cycle and the end-tidal part. For smokers, we can see a clear alveolar slope (visible in figure 2) and the bag measurements thus represent a mixture of the dead-space and end-tidal acetylene mixing ratios. The most important improvement could be obtained for the elimination kinetics measurements. The short-term irregularities observed in the decay curves would most likely be accounted for if the data were normalized in real-time to tidal volume and/or CO_2 concentration.

Acknowledgments

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