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Rapid communication

Real-time monitoring of ethane in human breath using mid-infrared cavity leak-out spectroscopy

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Abstract. We report on spectroscopic real-time analysis of ethane traces in exhaled human breath. Ethane is considered the most important volatile marker of free-radical induced lipid peroxidation and cell damage in the human body. Our measurements were carried out by means of mid-infrared cavity leak-out spectroscopy in the 3 μm region, a cw variant of cavity ring-down spectroscopy. The spectrometer is based on a CO overtone laser with tunable microwave sidebands. The resulting system proved to be an unique tool with high sensitivity and selectivity for rapid and precise breath testing. With a 5 s integration time, we achieved a detection limit on the order of 100 parts per trillion ethane in human breath. Thus, sample preconcentration is unnecessary. Time-resolved monitoring of the decaying ethane fraction in breath after smoking a cigarette is demonstrated.

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The analysis of trace components of human breath is an interesting, non-invasive way to in-vivo monitor various physiological and pathological processes in the human body. A current major biomedical research field is the investigation of free-radical-induced cell damage. The human body is continuously challenged by free radicals. They are endogenously generated or of exogenous origin. The overall equilibrium between formation and removal of free radicals is known as oxidative stress status [1]. Several factors are believed to affect the oxidative stress status of the human body such as hyperbaric oxygen, cigarette smoke, and ultraviolet light [2]. There is increasing evidence that cell damage by free radicals plays a crucial role in the pathogenesis of a number of chronic and acute diseases, such as cancer, inflammation, atherosclerosis, and Alzheimer's [3, 4]. Oxidative cell damage is related to lipid peroxidation, which is the free-radical-induced oxidative degradation of polyunsaturated fatty acids [5]. This process is ubiquitous in the human body, and therefore it became one of the the main targets in the investigation of oxidative damage of tissue [6]. Thus there is a need for methods to monitor in-vivo lipid peroxidation. Numerous methods have been developed for this monitoring. A widely used method in routine clinical praxis is the analysis of blood plasma for secondary reaction products of lipid peroxidation [7]. The only available non-invasive method is the analysis of appropriate volatile hydrocarbons, such as ethane (C_2H_6) and pentane (C_5H_{12}), in exhaled breath [4, 7]. These hydrocarbons are produced via a minor degradation pathway of lipid peroxidation, and they are poorly soluble in tissue. They are present in breath in volume fractions ranging from several hundred parts per trillion (ppt) up to several parts per billion (ppb). Ethane is considered to be the most specific volatile marker for non-invasive investigations of lipid peroxidation [2].

To date, the analysis of hydrocarbons in breath has usually been performed using gas chromatography (GC). Due to the insufficient sensitivity of this method, the breath sample, collected in bags, must undergo a trap-and-purge process to preconcentrate the sample before analysis. This procedure is very time consuming and prone to errors. Usually it takes hours to perform. For this reason, it has not become routine to monitor lipid peroxidation on a regular basis in the clinical praxis via non-invasive breath testing. These GC deficiencies also make it difficult and cumbersome to perform medical research, especially if a high time resolution is required.

Laser spectroscopic techniques show great promise for sensitive and selective analysis of hydrocarbons in human breath. Harren and coworkers have demonstrated that photoacoustic spectroscopy is sufficiently sensitive for on-line monitoring of breath ethylene (C_2H_4) [8]. Also, cavity ring-down spectroscopic techniques have proven to show extremely high sensitivities in the visible and near-infrared spectral region [9–11] as well as in the mid-infrared [12]. Our group has recently applied mid-infrared cavity leak-out (CALO) spectroscopy – a cw variant of cavity ring-down spectroscopy – to the precise, real-time detection of methane isotopomeres in ambient air [13, 14]. The sensitivity and time

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resolution achieved qualifies this technique for rapid real-time breath testing without preconcentration of the samples.

In this communication we report on the analysis of exhaled ethane via mid-infrared CALO spectroscopy. We used breath samples obtained from a smoker in a period of a few hours after smoking a cigarette. Smoking is known to have a strong influence on the ethane exhalation. Our main interest was focused on the question of whether the sensitivity and selectivity of the CALO spectroscopy method are sufficient to perform precise, time-resolved measurements of the ethane fraction in human breath.

1 Experiment

1.1 Spectrometer

The cavity leak-out spectrometer is described in detail in [14]. It basically makes use of a tunable CO-overtone sideband laser (wavelength range: $\lambda = 2.6–4.0\,\mu m$) which excites a high-finesse ring-down cavity (length: 53 cm).

The CO laser operates on about 300 rovibrational $\Delta v = 2$ transitions in the wavelength region between 2.6 and 4.0 μm with single-line output power on the order of 100 mW. By mixing the laser light with microwave radiation in an electro-optic modulator (EOM), tunable laser sidebands are generated covering a spectral range of 8 to 18 GHz above and below each laser line. The sideband radiation is mode matched to excite the fundamental transverse mode of the ring-down cavity. The cavity mirrors have a reflectivity of $R = 99.985\%$, which provides an effective optical absorption path length of more than 3 km. Frequency stabilization of the cavity resonance to the laser frequency is accomplished by means of a standard $1f$-lock-in technique (modulation frequency: 200 Hz).

In this way the laser power is periodically injected into the ring-down cell, twice per modulation period. Each time the transmitted light indicates optimum coincidence of laser frequency and cavity mode, a trigger pulse is provided to turn off the laser sideband radiation via the EOM. The subsequent leak-out of the cavity field is monitored with an InSb photodetector and acquired by means of a 12-bit analog-to-digital converter. The signal is typically averaged 100 times, which takes about 200 ms. The decay time (1/e time) of the leak-out signal is determined by fitting a single exponential to the data. By measuring the decay time of the empty cell and the decay time of the cell filled with the breath sample, the absorption coefficient can be directly determined.

To achieve selective ethane detection, we used a characteristic absorption line in the spectral region around $\lambda = 3\,\mu m$, where ethane shows a characteristic fingerprint spectrum. The appropriate spectral line was chosen by means of data from a high-resolution Fourier transform infrared (FTIR) spectrum of ethane which was recently acquired at the University of Bremen [15]. Use of this FTIR spectrum was essential, since the ethane-line positions provided by the commonly used HITRAN96 database [16] are not sufficiently accurate. We selected a characteristic ethane absorption peak near $3000.3\,cm^{-1}$, which can be reached by the upper frequency sideband of the P(10), $v' = 26 \rightarrow v'' = 24$ line of the CO overtone laser. To ensure high discrimination against interfering species such as methane, we reduced the pressure inside the absorption cell to 100 mbar, which eliminates most of the pressure broadening. The pressure was kept constant via an electronic pressure control loop.

1.2 Breath sample acquisition and handling

The breath samples being analyzed were obtained from volunteers in our group who regularly smoke. They agreed to provide a breath sample at certain intervals after smoking a cigarette. The volunteers were asked to hold their breath for 30 s after a normal inhalation and then exhale into a sample bag with a normal exhalation flow rate. The breath samples were collected in aluminum-coated bags of 1 L volume (Tecobag, Tesseraux GmbH) equipped with a valve that seals the bag after filling. Prior to the measurements, the sample bags were tested and they proved not to affect the ethane fraction of the breath samples. After filling, the sample bag was connected to the spectrometer, and the breath sample was analyzed. The period (for up to 2 d) between breath sample acquisition and analysis had no effect on the measurements.

For analysis of the breath sample a gas flow from the bag through the cell is maintained by a rotary pump. The gas flow is controlled by an electronic mass flow controller to be $100\,cm^3 min^{-1}$ at standard temperature (298 K) and pressure (1013 mbar) conditions. In order to minimize contamination by surface-released trace gases, all parts in contact with the gas flow are made of stainless steel, teflon or pyrex glass.

Human breath consists of many gases whose spectra may interfere with the ethane spectrum near 3 μm. Most difficulties arise due to water and methane. Moreover, the isoprene fraction in exhaled breath may be up to several 100 ppb [17], which could disturb a precise ethane measurement. To remove these components and all other heavier volatile compounds, a cooling trap (temperature: 120 K) is placed between the sample bag and the absorption cell. The fraction of all gases is thus reduced to their vapor pressure at that temperature. Only ethane, methane, and ethylene fractions remain unchanged. In order to prove that the cooling trap has no effect on the ethane fraction, we analyzed a controlled mixture of 300 ppb ethane in nitrogen before and after the cooling trap. In both cases our measurements yielded the correct ethane mixing ratio.

To make sure that only the endogenous ethane is measured, the ethane concentration of the ambient air which is inhaled has to be taken into account. This atmospheric ethane fraction may amount to several ppb, which is on the same order of magnitude as the endogenous ethane fraction in breath. This problem can be avoided by providing the proband with hydrocarbon-free medical air for a period of at least 5 min before the breath test [18]. This wash-out procedure reliably removes any ambient ethane from the lungs. Here, we followed a different approach. Shortly before the breath test we analysed the ethane fraction in the ambient air the proband was breathing. Therefore the proband had to be in our laboratory for at least 5 min before fill-

ing the sample bag. After the breath test, the ethane fraction of the ambient air is subtracted from the exhaled ethane fraction.

2 Results

2.1 Detection limit

In order to determine the instrumental detection limit for ethane, we analyzed a synthetic gas mixture consisting of trace amounts of ethane in nitrogen. This was accomplished by sending a gas flow of 13 $cm^3 min^{-1}$ of a certified gas mixture of 1 ppm ethane in grade 5 nitrogen mixed with a gas flow of grade 5 nitrogen at a flow rate of 210 $cm^3 min^{-1}$ at a pressure of 100 mbar through the absorption cell. This results in a fraction of 63 ppb ethane in the absorption cell. Figure 1 shows the ethane spectrum obtained. The laser sideband frequency was tuned in steps of 142.5 MHz. Each data point represents the mean of 5 consecutive decay time measurements, which were acquired from 100 averages of single ring-down events. The total measurement time for each data point in Fig. 1 was approximately 2 s. For comparison, the FTIR spectrum – scaled to the corresponding low concentration – is displayed as a solid line. It should be noted that the spectral resolution of the FTIR spectrum is 0.005 cm^{-1}, which is about 2 orders of magnitude worse than the resolution of our CALO measurements. Despite the lower spectral resolution of the FTIR data, the agreement between our data and the FTIR data is satisfying. The error bars include the statistical uncertainties of the absorption measurement and of the τ_0 measurement of the empty cell decay time. Not included are systematic errors due to the uncertainty of the conversion from the measured absorption coefficient to the corresponding volume fraction of the gas. We estimate this absolute uncertainty to be less than 5%. Under optimum conditions the detection limit is about 100 ppt ethane (integration time: 5 s), corresponding to a minimum detectable absorption coefficient of 1×10^{-9} cm^{-1}.

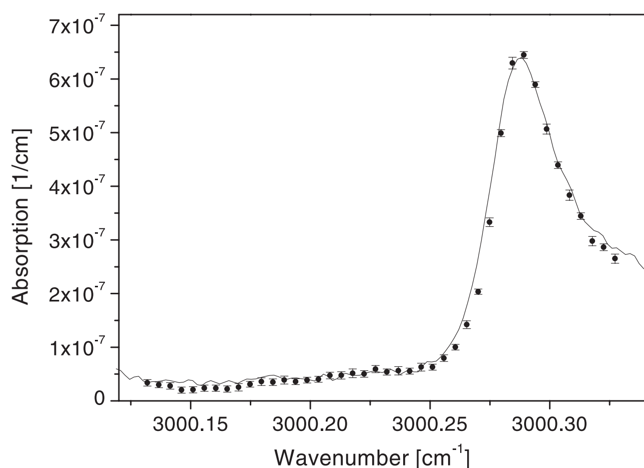

Fig. 1. Observed CALO spectrum of ethane near 3000.3 cm^{-1}. The absorption cell contained a gas mixture of 63 ppb ethane in nitrogen (pressure: 100 mbar). This ethane peak served as a fingerprint for the subsequent ethane breath tests. *Data points*: experimental. *Solid line*: data from an FTIR ethane spectrum (spectral resolution: 0.005 cm^{-1}), scaled to the corresponding concentration

Due to drifts and statistic fluctuations of the background signal, i.e., the decay time of the empty absorption cell, the long-term sensitivity (minutes to hours) is slightly worse but still allows for the detection of ethane fractions down to 500 ppt.

2.2 Selectivity

In the spectral region we chose, methane is the only interfering species which is not removed by the cooling trap. Fig. 2 shows the CALO spectrum of a breath sample containing a relatively large fraction of methane. The dotted line shows the methane spectrum calculated from the HITRAN96 database. The methane spectrum shows two weak absorption lines at the position of the ethane line used for the breath test. These methane lines cannot be separated from the ethane line by pressure reduction. To take this into account, the methane fraction of the breath sample was simultaneously analysed by a measurement of the isolated methane line at 3000.172 cm^{-1} (see Fig. 2). The HITRAN database provides the corresponding concentration of methane. Using this, the absorption strength of the interfering methane lines are determined and subtracted from the measured spectrum.

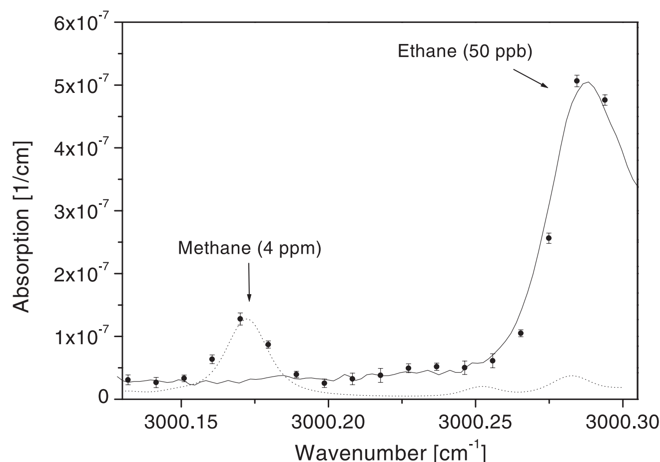

Fig. 2. Observed CALO spectrum of a breath sample (pressure: 100 mbar). The *left arrow* indicates the methane line that was used for the methane correction of the ethane measurements. *Data points:* experimental. *Solid line:* data from a FTIR ethane spectrum. *Dotted line:* HITRAN96 data for methane

2.3 Ethane exhalation after smoking

In order to demonstrate the application of our CALO spectrometer for precise time-resolved breath testing, we monitored the ethane fraction exhaled by a smoker in a period of several hours after smoking a cigarette. The proband (29-yr-old male) smokes a cigarette approximately every 45 min during the day. We took breath samples about every 30 min in a period of 4 h after smoking. During this 4-h period the proband volunteered not to smoke. The first breath sample was not taken until a 30 min waiting period after the cigarette was finished. This ensured that no cigarette smoke was present in the lungs. Figure 3 shows the results. The

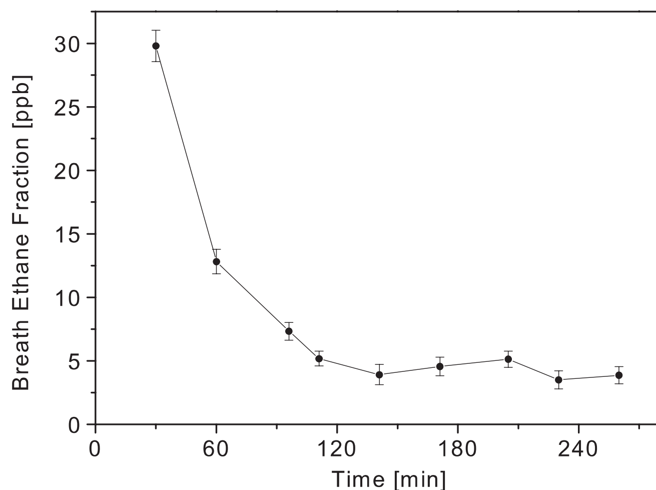

Fig. 3. Typical decaying time course of the breath ethane fraction observed after smoking. The cigarette was smoked at $t = 0$

measurements of the data points displayed include several absorption measurements on top of and next to the ethane line; this took about a total of 1 min for each data point. The uncertainty of each data point is about 1 to 2 ppb, including the statistical error of the consecutive absorption measurements and the error introduced by the background correction.

The measurements shows a decaying ethane exhalation in a period of 2 to 3 h after smoking, depending on the proband. As discussed by Habib et al. [19], who carried out gas chromatographic ethane measurements with smokers, the high ethane fraction shortly after smoking is probably caused by the oxidative damage of epithelium cells in the respiratory tract due to the large amount of free radicals in cigarette smoke. A part of the exhaled ethane could also originate from the cigarette. Since cigarette smoke itself contains ethane, a fraction of this may be stored in the body after inhalation and then slowly released afterwards.

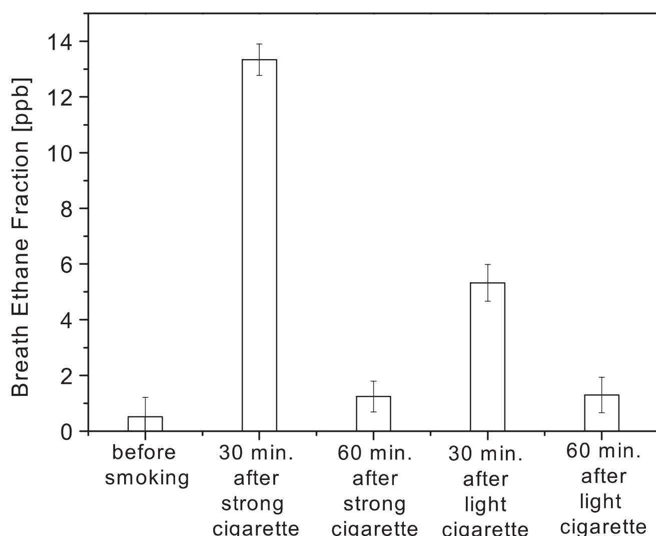

Fig. 4. Observed influence of the cigarette brand on the ethane expiration. From left to right: before smoking, 30 min after smoking a strong cigarette, 1 h after smoking a strong cigarette, 30 min after smoking a light cigarette, 1 h after smoking a light cigarette

Even though it was not the goal of our study to investigate the origin of exhaled ethane with smokers, we became interested as to whether the ethane fraction of breath depends on the strength of the cigarette smoked. We asked an irregular smoker to smoke two different cigarette brands of different strength. We found that the exhaled ethane fraction is significantly increased when a stronger cigarette is smoked. The ethane fraction was measured 30 min after smoking in both cases. Figure 4 shows the results. Smoking of a strong cigarette (0.9 mg nicotine, 12 mg tar condensate) results in an exhaled ethane fraction which was twice the level observed after smoking a light cigarette (0.6 mg nicotine, 9 mg tar condensate). These results were reproducible independent of the order in which the strong and light cigarettes were smoked. One hour after smoking, the ethane fraction had dropped to about 1 ppb, independent of the cigarette brand.

These measurements certainly should not be considered to constitute a systematic study. However, our results demonstrate that the CALO spectroscopy method is well suited for such investigations.

3 Conclusion

In conclusion, our work demonstrates that CALO spectroscopy is an excellent method for the rapid and precise analysis of ethane fractions in human breath. By achieving a detection limit of 500 ppt, we could perform real-time monitoring of exhaled ethane in the breath of smokers. Our measurements show decaying ethane exhalation 1 to 3 h after smoking. This decay is qualitatively in agreement with the results of Habib et al., who carried out chromatographic ethane measurements with smokers [19]. Compared with gas chromatography techniques the data acquisition is very fast, since time-consuming preconcentration of the breath sample is not necessary. This is major progress in regard to facilitating the use of breath tests in clinical research and praxis.

To our knowledge there is no spectroscopic method with a higher sensitivity for ethane and a sufficiently low cross sensitivity of other gases, such as methane, ethylene, etc. Detection of ethane in synthetic gas mixtures by means of photoacoustic spectroscopy was recently reported to have a comparable sensitivity [20, 21]. However, since photoacoustic spectroscopy has to be carried out at ambient pressure, leading to considerably pressure-broadened spectral lines, it is difficult to achieve a satisfying selectivity when analyzing breath samples.

The presented approach has the potential to become a versatile tool for non-invasive monitoring of in-vivo lipid peroxidation and for other breath tests based on exhaled trace gases. An important condition for acceptance in the medical community is the mobility of the instrument. We have currently completed a portable spectrometer, where the bulky CO laser is replaced by a compact, tunable difference-frequency generation setup. This will be published elsewhere.

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