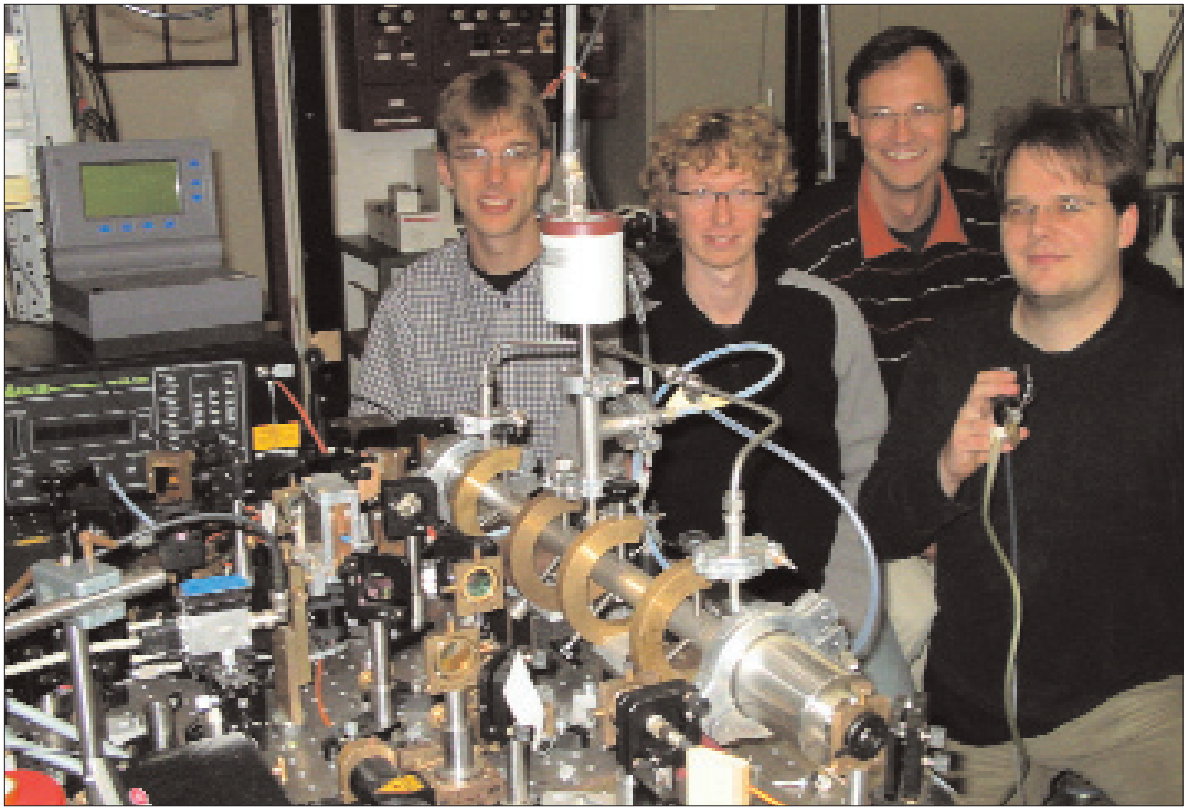


Breath Diagnostics
Using Laser Spectroscopy

Manfred Mürtz

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(*Left to right*) Markus Horstjann, Golo von Basum, Manfred Mürtz and Daniel Halmer.

This article describes the merits of laser-assisted analytical instrumentation for biomedical diagnostics, in particular for the analysis of exhaled breath. The characteristics of laser spectroscopic techniques for breath monitoring are discussed and potential applications of human breath tests based on laser spectrometers are outlined.

Breath tests have a long history in medical diagnostics. The fact that the breath of patients suffering from certain diseases is characterized by a specific odor has been well known since the time of Hippocrates: the fruity aroma of acetone, for example, is related to untreated diabetes, while the fishy smell of sulfur compounds is an indication of liver impairment. Modern breath analysis began with the discoveries of Antoine Laurent Lavoisier (1743-1794), who identified carbon dioxide as a major constituent of exhaled breath in the late 1780s. More recently, in 1971, Nobel Laureate Linus Pauling achieved a milestone in breath analysis when he identified a large number of volatiles in human breath. Pauling breathed through a very cold tube ($T < 200$ K) and then analyzed the frozen compounds by means of gas chromatography. He found that normal human breath contains a number of volatile organic compounds (VOCs) in very low concentrations. Today we know that the VOCs found in breath are predominantly bloodborne and are exhaled via the blood/breath interface in the lungs. Several hundred VOCs, as well as some diatomics and triatomics of endogenous origin, have so far been identified in exhaled breath. Most of them are present in picomolar (10^{-12} mol/l) to nanomolar (10^{-9} mol/l) concentrations.

The analysis of breath metabolites could open new diagnostic windows in clinical medicine. Breath analysis is a particularly attractive diagnostic tool in that it is a noninvasive way to monitor a patient's physiological status. Detection of compounds in such low concentrations does, however, remain a difficult analytical problem. Today, gas chromatographic and mass spectrometric methods are usually applied to separate and identify trace gases in breath.[1] In this article, I describe modern laser-based analytics which allow measurements to be taken with unprecedented sensitivity, specificity and speed: trace gas fractions down to picomolar levels can be quantitatively analyzed in real time with subsecond resolution.

The components of breath

Besides its primary constituents—nitrogen, oxygen and water—exhaled human breath contains various volatile metabolites that are formed in the body. The major metabolite in breath is carbon dioxide (CO_2), which is exhaled in a fraction equal to about 4 percent of volume. Although CO_2 in itself is not a disease marker, it plays an important role in breath testing when ^{13}C-labeled pharmaceuticals are applied. A prominent example of isotopic breath testing based on carbon dioxide is the noninvasive

verification of *Helicobacter pylori* infection in the gastrointestinal tract by means of a $^{13}CO_2/^{12}CO_2$ breath test. This is one of the few diagnostic breath tests to have been approved so far by the U.S. Food and Drug Administration (FDA).

The primary endogenous trace gases found in exhaled breath are shown in Table 1. Although many other compounds, in particular VOCs, have also been identified, a large number are of exogenous origin and have just been either ingested or inhaled with polluted ambient air. A good example is ethanol. The presence of such volatiles in breath is not evidence of disease but rather an indicator of recent exposure to the compound in question.

Although it has been theorized for many years that some breath VOCs are markers of disease, verification of the hypotheses has been slow in most cases, primarily because it is very difficult to reliably analyze ultralow concentrations by means of conventional instrumentation. Another challenge in this respect has been the shortage of knowledge about the way in which volatile metabolites are synthesized. A few exhaled trace compounds have, however, been investigated quite thoroughly. Examples are nitric oxide and small hydrocarbons, such as alkanes. There is strong evidence that the analysis of these and other trace constituents in exhaled breath could provide a new, noninvasive way to monitor inflammation, oxidative stress and other processes in the airways and lungs.[2]

The most prominent disease marker in exhaled human breath is nitric oxide (NO). Little more than a decade ago, nitric oxide was regarded primarily as a noxious gaseous component of air pollution. Since that time, intense basic research and clinical investigation have shown that nitric oxide is produced by a variety of human tissues. Nitric oxide is now known to be a central mediator in biological systems. The presence of endogenous NO in the exhaled breath of animals and humans was first described by Gustaffson and coworkers in 1991. The average fraction of exhaled nitric oxide (eNO) is generally in the low nanomolar region and can be analyzed by means of a chemiluminescence technique. Nitric oxide is the most extensively studied exhaled marker and abnormalities in eNO have been documented in several lung diseases, particularly asthma.[3] When the airways are inflamed, as is the case in patients with asthma, NO concentrations in exhaled air are significantly increased. The NO breath test to monitor inflammation in asthma has recently been given FDA approval as a diagnostic tool.



Figure 1. Hippocrates considered the odor of exhaled breath an important tool in diagnosing disease.

Among the various volatile hydrocarbons found in breath, the alkanes ethane (C_2H_6) and pentane (C_5H_{12}) have been extensively studied since their identification as end-products of the oxidative degradation (lipid peroxidation) of polyunsaturated fatty acids. As one of the important features of free-radical-induced damage of tissues, the process of lipid peroxidation has attracted interest among researchers in biology and medicine. Ethane is formed during the peroxidation of the omega-3 fatty acid; pentane is formed during the peroxidation of the omega-6 fatty acid. Ethane and pentane are excreted via the lungs and can thus be detected in exhaled breath. Enhanced free-radical-induced damage, which is known as oxidative stress, has been related to a variety of diseases, including diabetes mellitus, rheumatoid arthritis and chronic obstructive pulmonary disease. Since lipid peroxidation is considered at least the major—if not the only—endogenous source of pentane and ethane, these volatile compounds could serve as specific markers of oxidative damage.

Breath constituent	Average fraction
Methane (CH_4)	2 – 10 ppm
Ethane (C_2H_6)	0 – 10 ppb
Pentane (C_5H_{12})	0 – 10 ppb
Nitric Oxide (NO)	10 – 50 ppb
Carbon Monoxide (CO)	1 – 10 ppm
Carbonyl Sulfide (OCS)	0 – 10 ppb
Nitrous Oxide (N_2O)	1 – 20 ppb
Isoprene (C_5H_8)	50 – 200 ppb
Ammonia (NH_3)	0 – 1 ppm
Acetone ($(CH_3)_2CO$)	0 – 1 ppm

Table 1. Selection of endogenous trace gases found in exhaled breath and their average concentration in the breath of healthy humans.

A large number of other molecules have been found in exhaled air, but there is little information about how and in what amounts these compounds are excreted in the breath; in some cases, it is not even clear whether the compounds in question are endogenous or not. Various volatiles stemming from the inhalation of polluted air are interesting as markers of exposure to toxic compounds. The fact that breath tests can be used to measure exhaled markers noninvasively makes them ideal for serial monitoring of patients. In this context, the sampling and analysis of breath are preferable to direct measurement of the metabolites from blood samples. What's more, measurements are much simpler in gas than in a complex biological fluid such as blood or urine.

Using lasers to detect breath markers

The development of rapid, sensitive analysis techniques to measure the volatile compounds released in exhaled breath remains a challenge. Since conventional methods such as gas chromatography and mass spectrometry barely reach nanomolar sensitivity, they cannot enable reliable monitoring of many exhaled VOCs. Optical detection techniques, on the other hand, have proven to be suitable for sensitive, specific and fast detection of many hydrocarbons and other volatile compounds in atmospheric air.

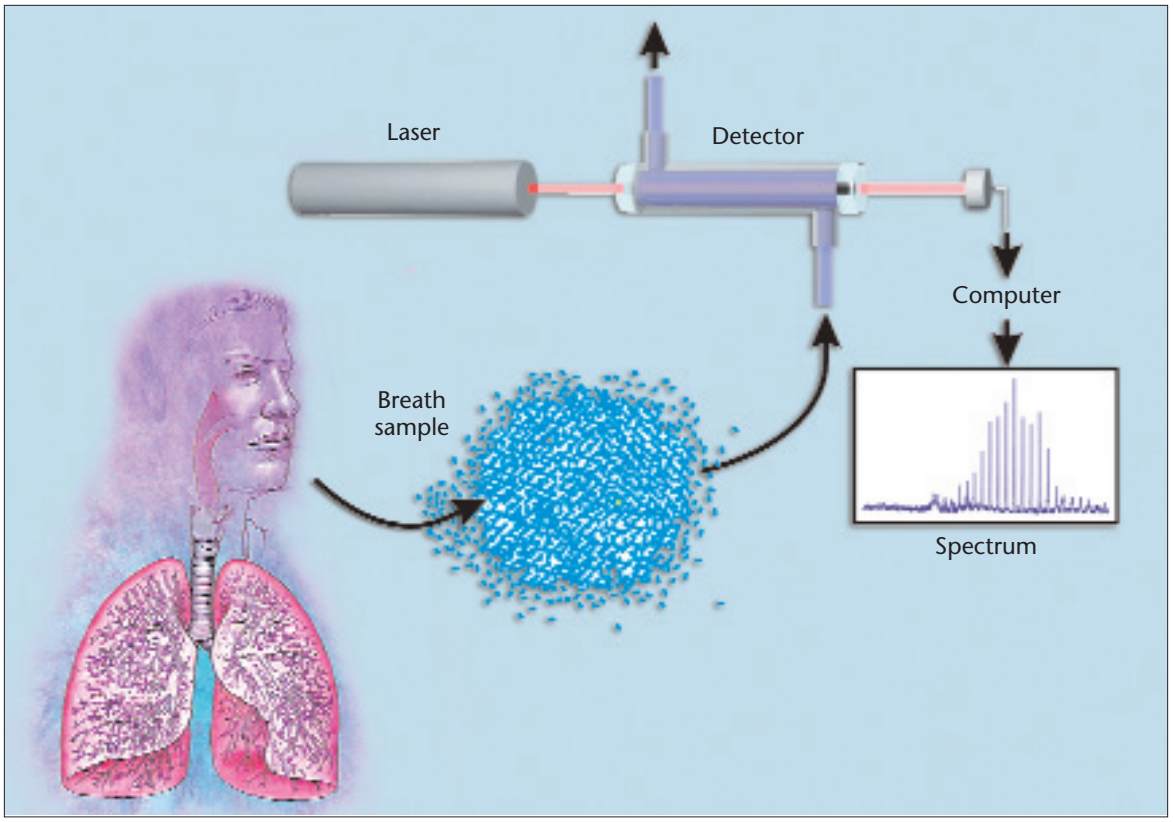


Figure 2. The lungs are a huge blood-air interface where volatile blood-borne metabolites transition to the gas phase before being excreted with exhaled air. Laser absorption spectroscopy is based on the measurement of wavelength-dependent attenuation of laser light after it passes through the gas sample in the absorption cell.

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Optical methods can be used to record a characteristic spectral fingerprint of the molecular compounds. Most of the optical methods are based on absorption spectroscopy: the gas sample of interest is transferred into an absorption cell and the wavelength-dependent attenuation of the laser light which passes through the cell is measured. This is shown in Fig. 2. The mid-infrared region ($\lambda = 3\text{-}10\ \mu m$) is of particular interest for this purpose since the fundamental vibrational transitions of most molecules lead to strong absorption lines in this spectral window.

A major advantage of laser spectroscopic breath analysis is that it makes possible online measurement in the context of real-time analysis during exhalation. With offline techniques, breath is collected in a bag or sorbent trap in a process that can be characterized by problems regarding the reproducibility of breath sample collection, contamination during sample storage and the impossibility of obtaining instantaneous feedback. Online methods are free of such drawbacks. In addition, fast online measurement makes it possible to access information about concentration during different phases of exhalation; offline methods integrate information obtained over the course of a complete exhalation or require an extra effort to separate exhaled gas coming out of the lungs from gas that originates in the upper airways (dead space air).

The excellent properties of laser radiation, including spectrally narrow emission and low intensity fluctuations, have enabled the development of analytical techniques with high sensitivity and specificity. In the past decade, significant progress has been made in the development of laser spectroscopic methods for analytical use.[4] For example, photoacoustic spectroscopy and cavity ring-down spectroscopy (CRDS) have achieved ultrahigh sensitivity: detection limits in the picomolar region have been demonstrated. Additionally, progress in laser technology has enabled the development of compact, high-performance infrared laser sources with outstanding spectral properties, i.e., narrow linewidth, wide wavelength tunability and smooth beam profiles. Suitable coherent light sources for mid-infrared spectroscopy include semiconductor lasers, such as quantum cascade lasers (QCL), as well as all-solid-state pumped nonlinear conversion devices based on difference frequency generation (DFG) or optical parametric oscillation (OPO).

To achieve high detection sensitivity, most laser absorption spectrometers are equipped with a multi-pass absorption cell. This type of absorption cell enhances sensitivity because it allows multiple passes (up to 100) of the laser beam through the gas sample, thus extending the path of interaction between laser beam and molecules. CRDS is a special variant of laser absorption spectroscopy

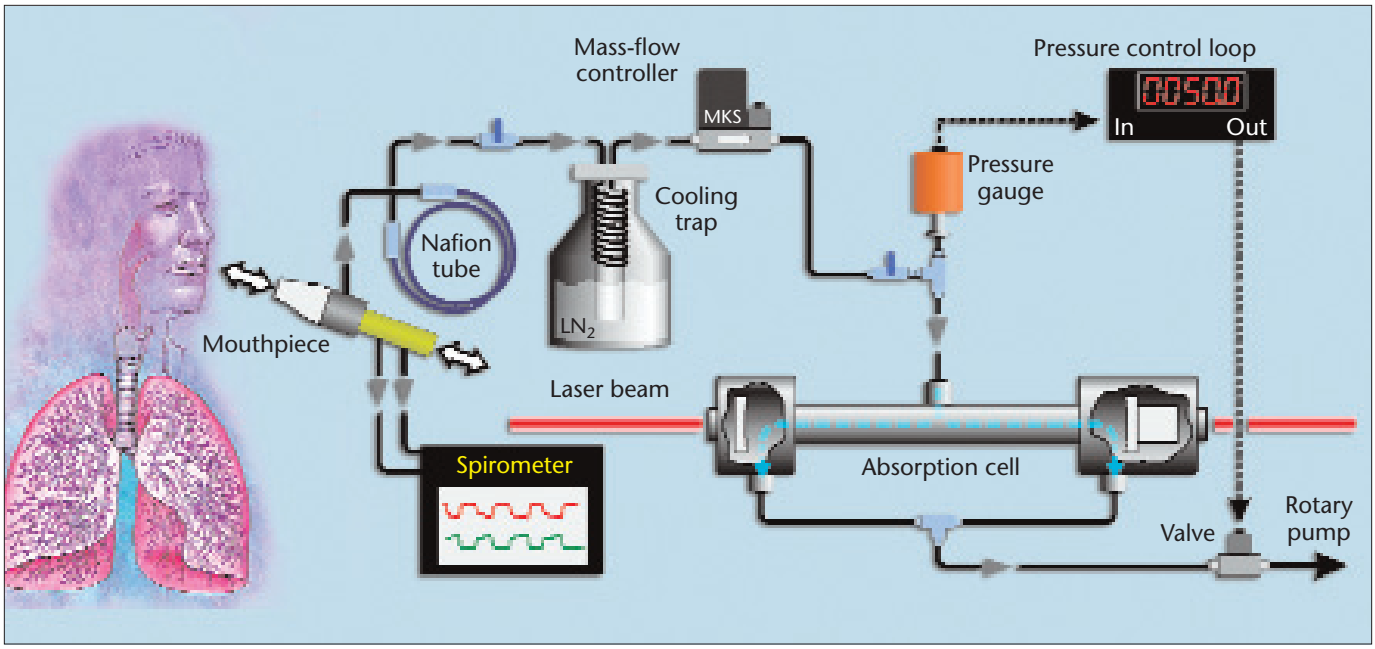


Figure 3. For online recording of breath metabolites, part of the exhaled breath is transferred from a mouthpiece to the absorption cell.

Laser spectroscopy is currently the only technique that allows for breath-resolved online measurements of exhaled traces gases with picomolar sensitivity.

which, in the past decade, has revolutionized the sensitivity and speed of trace gas analysis.[5] It is based on the optical excitation of a high-finesse cavity which encloses the gas sample to be analyzed. The absorption measurement is carried out via monitoring of the decay rate of the light leaking out of the cavity. The very long effective path length (several kilometers) translates directly into ultrahigh sensitivity. Because of its ultrahigh sensitivity and extraordinary specificity, CRDS is perfectly suited to measure the trace levels of the volatile biomarkers present in exhaled human breath.

Our group at the Institute for Laser Medicine at the University of Düsseldorf has recently demonstrated that CRDS is capable of online quantification of ethane traces in exhaled human breath down to 500 ppt (parts per trillion) with a time resolution of better than 800 ms.[6] Part of the exhaled breath is transferred to the absorption cell, as shown in Fig. 3. The nafion tube and cooling trap are used to eliminate extraneous compounds, such as water and carbon dioxide, from the breath sample. An example of an online recording obtained in this way is shown in Fig. 4. The top curve displays the course of the breath ethane concentration detected with the laser spectrometer. For demonstration purposes, before the recording started, a volunteer inhaled 1 ppm ethane in synthetic air for a duration of 5 min., thus enriching the organism with ethane. The curves in the lower part of the figure show the concentration of breath CO_2, O_2 and flow as analyzed by means of a capnograph/spirometer. The ambient air concentration of ethane was constant in the low ppb range (ppb = 10^{-9}) during each experiment and was subtracted from the measured exhaled breath fraction.

One aspect of this work was to measure the profile of a single exhalation. In this case, the recorded concentrations were plotted against the exhaled volume (expirogram), which was calculated by integrating the expiratory flow. Typical expirograms are shown in Fig. 5. The expirograms for ethane, obtained from the single breath analysis, show a shape similar to that of the CO_2 expirograms. At the beginning of expiration, the exhaled air comes from the trachea and the bronchioles, where there is no gaseous exchange between the blood and the air (phase I, "dead-space" air). In phase II, a fast increase is observable. Finally a plateau is reached (phase III), in which the alveolar breath, which has undergone gaseous exchange with the blood, is exhaled. Interestingly, we found an additional increase in ethane concentration if the test subject exhaled beyond his/her functional residual volume (phase IV). This additional increase in concentration was not observed in either the CO_2 or the O_2 curve. These data constitute dramatic evidence that laser spectroscopic breath analysis opens new diagnostic windows.

Prospects of breath diagnostics with lasers

Breath diagnostics is still in its infancy, in the sense that there is little or no knowledge of the origin and role of most exhaled metabolites. Since the exhaled concentrations are so low, the technical problems inherent in breath testing are substantial. Laser spectroscopic analysis offers exciting opportunities since laser spectroscopy is currently the only technique that allows for breath-resolved

online measurements of exhaled trace gases with picomolar sensitivity. In particular, the sensitivity and specificity (e.g., for breath ethane monitoring) are unprecedented.

There are numerous potential biomedical applications for online breath analysis instruments. One important example is bedside monitoring of ischemia-reperfusion injury after extracorporal blood flow. Significant oxidative stress and related critical tissue damage are associated, for example, with reperfusion after a long period of ischemia that takes place after cardiothoracic surgery or organ transplant. In such situations, laser spectroscopic online monitoring of breath ethane could serve as a noninvasive means of acquiring information on oxidative stress status. In any case, the clinical value of exhaled biomarkers will depend on the availability of reliable, fast and inexpensive detector systems. Advances in optical technologies will soon result in smaller devices which are cheaper and easier to use. Recent innovations in infrared lasers and spectroscopic instrumentation may enable laser-based analytical instruments to be deployed in commercial environments, such as doctors' offices or clinics, in the near future.

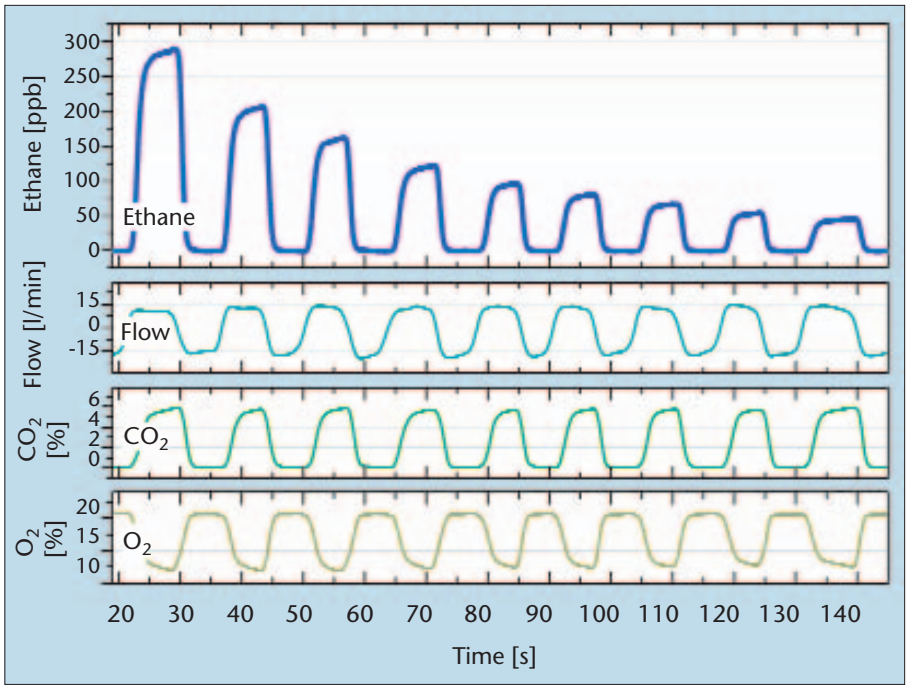


Figure 4. Simultaneous online recording of exhaled ethane, flow carbon dioxide and oxygen. The ethane curve was measured with an infrared cavity ring-down spectrometer.

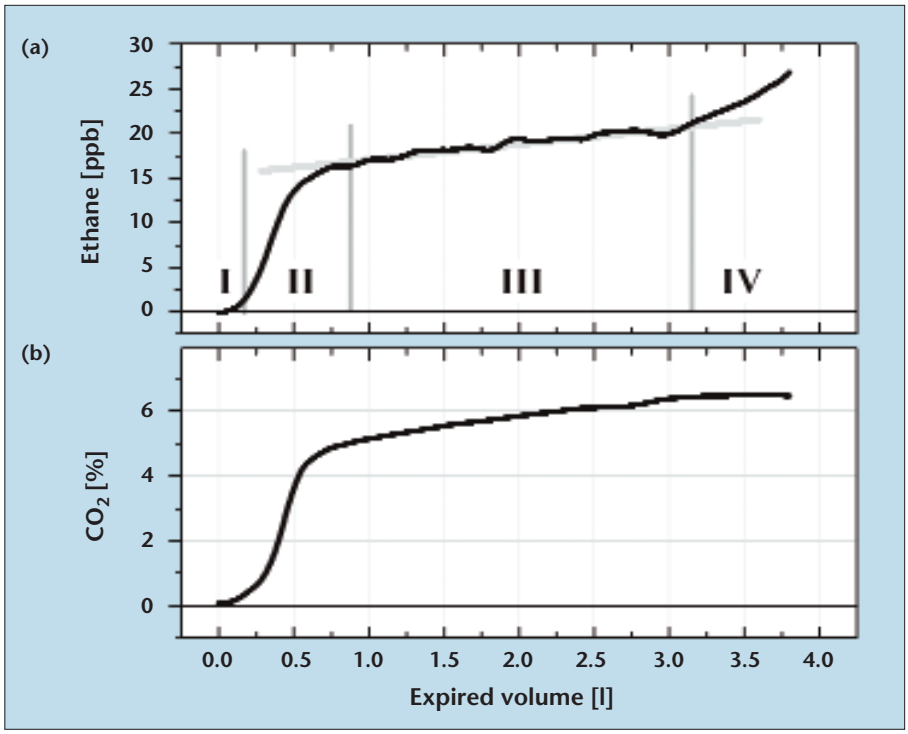


Figure 5. (a) Exhaled ethane over exhaled volume. The graph shows four different phases of exhalation. (b) Exhaled carbon dioxide profile of the same exhalation.

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