Intracavity CO laser photoacoustic trace gas detection: cyclic CH$_4$, H$_2$O and CO$_2$ emission by cockroaches and scarab beetles

F. G. C. Bijnen, F. J. M. Harren, J. H. P. Hackstein, and J. Reuss

A liquid-nitrogen-cooled CO laser and an intracavity resonant photoacoustic cell are employed to monitor trace gases. The setup was designed to monitor trace gas emissions of biological samples on line. The arrangement offers the possibility to measure gases at the $10^9$ by volume (ppbv) level (e.g., CH$_4$, H$_2$O) and to detect rapid changes in trace gas emission. A detection limit of 1 ppbv for CH$_4$ in N$_2$ equivalent to a minimal detectable absorption of $3 \times 10^{-10}$ cm$^{-1}$ can be achieved. Because of the kinetic cooling effect we lowered the detection limit for CH$_4$ in air is decreased to 10 ppbv.

We used the instrument in a first application to measure the CH$_4$ and H$_2$O emission of individual cockroaches and scarab beetles. These emissions could be correlated with CO$_2$ emissions that were recorded simultaneously with an infrared gas analyzer. Characteristic breathing patterns of the insects could be observed; unexpectedly methane was also found to be released. © 1996 Optical Society of America

Key words: CO laser, photoacoustics, trace gas, methane, water, insects.

1. Introduction

Infrared photoacoustic (PA) spectroscopy is one of the most sensitive techniques that one can use to measure low trace gas concentrations under atmospheric conditions.$^1$ CO$_2$ lasers have been employed for excitation predominantly because of their high output power in the infrared (900–1100 cm$^{-1}$) and their relative ease of operation. Optimizing the PA signal can be achieved by the cell design and intracavity laser operation. Other laser sources employed to detect photoacoustically atmospheric trace gases are He–Ne$^{2,3}$ and diode lasers.$^4$ In comparison the CO$_2$ laser possesses broad tunability and high laser power permitting measurements of mixtures of several gases at (sub)-part per billion volume concentrations (1 part per billion = $10^{-9}$).$^5$

The conditions for high laser power and broad line tunability are also met by a CO laser. In particular the liquid-nitrogen-cooled ($\Delta V = 1$) CO laser is line tunable over a large frequency range (350 lines between 1200 and 2100 cm$^{-1}$)$^6$, in which many gases possess a strong fingerprint absorption for their fundamental vibration modes. Furthermore, it is also possible to use the CO laser on $\Delta V = 2$ (300 laser lines), thereby reaching the range from 2450 to 3800 cm$^{-1}$.$^9$

Previously,$^{10}$ a cw CO laser was employed in a PA arrangement to monitor car exhaust gases. Multiline emission and water vapor absorption generated problems and made it necessary for one to use a dual-beam arrangement with a reference system. To avoid multiline emission the laser must be cooled from the 265 K used by Bernegger and Sigrist$^{10}$ to a liquid-nitrogen temperature of 77 K.$^{11}$ This yields higher population densities, resulting in laser action for the lower $J$ levels from $P(6)$ to $P(13)$ compared with the 265 K CO laser that functions mainly between $P(13)$ and $P(24)$. The rovibrational population is distributed over fewer and lower rotational levels, which causes less overlap between adjacent vibrational transitions resulting in single-line lasing conditions for almost all laser lines. Since the population density is higher for the lower-lying rotational levels, the population difference between the lasing levels is also larger. Therefore, laser action occurs even at weak vibrational transitions, yielding...
an extended wavelength range from 1260 cm$^{-1}$ ($V = 35 \rightarrow 34$) to 2080 cm$^{-1}$ ($V = 3 \rightarrow 2$).$^{6-8}$

We focus on the performance and characteristics of the liquid-nitrogen-cooled CO laser and an intracavity PA cell. We optimized the PA cell to detect fast changes of low production sources of CH$_4$ and H$_2$O. The experimental setup is applied to monitor trace gas release from single insects.

Atmospheric CH$_4$ contributes significantly to the process of global warming and ozone depletion. Biogenic sources are responsible for the major part of the mondial CH$_4$ flux ($>70\%$); the contribution of each different source is still a question for debate.$^{12}$ Some arthropods (i.e., millipedes, cockroaches, termites, and scarab beetles) are believed to contribute as much as 25% of the total production.$^{13}$ Gas chromatography allows measurement of the methane release of single animals only after incubation for several hours. Consequently these measurements cannot provide information about the dynamic character of the gas emission. Infrared absorption measurements yield fast response times and permit observation of the dynamics of CO$_2$ emissions.

In addition to CH$_4$ and CO$_2$ emissions of insects, water vapor release has been the subject of many studies. Information about water loss dynamics in insects is based on theoretical modeling, sensitive weighing, and relatively slow direct water vapor measurements.$^{14-17}$

Cockroaches at rest show a regular breathing pattern that is postulated to reduce water loss and optimize O$_2$ uptake.$^{14}$ This breathing is accomplished by a complicated sequence of opening and closing of the spiracle valves of the tracheal system and by venting the content of the tracheae through abdominal motions. We monitored the H$_2$O and CH$_4$ release, together with the CO$_2$ emission, during the complete sequence of fluttering, ventilation, and constriction for the cockroach species Periplaneta americana and Gromphadorhina portentosa and for the scarab beetle Pachnoda bhutana.

2. CO Laser

Inside the plasma discharge of the CO laser, inelastic electron scattering produces states of excitation of low-lying vibrational N$_2$ and CO molecules. By collisional transfer of vibrational energy from N$_2$ to CO the population of the lower vibrational states of carbon monoxide is further enhanced. Because of the vibrational anharmonicity of the electronic ground state, $X^2\Sigma$, the spacing of the vibrational levels decreases as $V$ increases. By energy pooling collisions CO molecules are therefore pushed further up the vibrational ladder. Because of this V–V or Treanor pumping one can reach high-lying vibrational levels.$^{18}$ The pumping is more effective when the translational temperature of the discharge is low. The rotational temperature is closely coupled to the translational temperature but the vibrational population is entirely athermal and possesses the so-called Treanor plateau ($V = 7$ to $V = 36$). We can assign an effective temperature only to parts of the vibrational distribution. For the Treanor plateau a typical temperature of 20,000 K can be ascribed.$^{19}$ Consequently partial population inversion can occur; the higher vibrational state ($V + 1$) with the lower rotational quantum number ($J = 1$) is more populated than the ($V, J$) state. Corresponding $P$-type transitions are observed to show laser action.

The experimental arrangement is shown in Fig. 1. With some minor changes the liquid-nitrogen-cooled CO laser tube is based on the design of Wu et al.$^{8}$ and has been constructed in our workshop. When the gas mixture was optimized for an optimal laser power on a weak laser line [$P(10)_{32}, 1304.97$ cm$^{-1}$] laser operation was achieved between 1260 and 2000 cm$^{-1}$ on 250 lines in one single scan (see Fig. 2). Intracavity laser power amounts to 40 W (1920 cm$^{-1}$). In the long wavelength region of the emission spectrum

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Fig. 1. CO laser detection setup in combination with a PA cell: 1, grating to select the appropriate transition; 2, power meter at zero-order reflection of the grating; 3, PA cell; 4, inlet for trace gas running through a quarter lambda notch filter; 5, resonator tube; 6, buffer volume; 7, tunable side arm to minimize window signal; 8, inlet for buffer gas; 9, outlet for buffer gas and trace gas; 10, microphone; 11, CO laser; 12, helium flow to flush the windows; 13, liquid-nitrogen jacket; 14, He, CO, N$_2$, and air mixing barrel in front of laser gas inlet; 15, laser gas outlet toward pump; 16, N$_2$ flow to start laser discharge at start of measurement; 17, chopper; 18, 100% reflecting mirror ($R = 10\%$); 19, cuvette containing insect; 20, infrared gas analyzer (URAS); 21, 22, cooling traps to remove water vapor for CH$_4$ measurements.

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Fig. 2. 250 CO laser lines between 1260 and 2000 cm$^{-1}$ (7.7 and 5.0 μm). Note that lines around 1750 cm$^{-1}$ are missing because of water vapor absorption.
Reflecting mirror provided a rigid mount for the grating and the 100% temperature at the flow rates applied. Invar rods were sufficient to cool the gas to liquid-nitrogen temperature. Our experiments showed that a length of 40 mm would have been approximately 0.7 m long, however a simple extension thermally shielded by a vacuum jacket. The laser was approximately 1.3 m long, surrounded by a liquid-nitrogen bath 1.16 m was surrounded by a liquid-nitrogen bath.
Inside the PA cell a longitudinal resonator optimally sustained this ensuing periodic pressure modulation and a condenser microphone mounted at the antinode of the resonator was used to monitor the sound. The resonator was acoustically separated from the windows by buffers. Depending on the length and radius of the resonator this type of configuration leads, in general, to a high cell constant \((10^3–10^4 \text{ Pa cm/W})\). The cell constant is a measure for the sensitivity expressed as the acoustic pressure amplitude resulting from a normalized quantity of energy absorbed over the length of 1 cm by the trace gas. Notwithstanding the high sensitivity a relatively low \(Q\) factor results ranging between 10 and 100. To avoid temperature drifts of the cell that are due to replenishment of the laser with liquid nitrogen, by use of a control unit we maintained the cell temperature at 23 °C, which is just above the temperature of the laboratory.

The PA cell was placed inside the CO laser cavity to profit from the order of magnitude power increase compared with an extracavity position. The cell was positioned close to the grating near the minimum waist of the laser beam; the 100% reflecting silver-coated mirror \((R = 10 \text{ m})\) was used to focus the beam on the grating. The frequency stability demands were easily met by our chopper (0.2%). For intracavity laser operation an asymmetric blade (60% open) was employed. The chopping frequency corresponded to an acoustic wavelength of twice the length of the resonator. The chopper positioned at the mirror side of the laser cavity produced sound and mechanical vibrations coherent with the modulated infrared radiation. These coupled through the Invar rods and the body of the PA cell and yielded a constant background signal. We reduced the coupling by maximizing the distance of the chopping blade to any object in its vicinity. With the blade mounted to the driving motor axis one could turn it freely in space without acoustic shielding. We obtained a further reduction of coupling by mounting the chopper and PA cell onto vibrational dampers that were attached to the Invar rod frame. At the acoustic resonance of the PA cell, this resulted in a constant background signal of 3 \(\mu\text{Pa}\), which is just above the detection limit of the detector.

The dimensions of the resonator and the resulting acoustic characteristics of the cell are listed in Table 1. For the acoustic resonator we chose a small radius, which is preferable since it results in a higher cell constant.\(^{21}\) A radius of 6 mm proved to be too small; an unstable background signal was observed that was due to wall heating by the wings of the Gaussian beam. We reached a good compromise by increasing the resonator radius to 7.5 mm. Choosing a long resonator length results in a higher cell constant and would be advantageous. On the other hand, a short resonator length reduced the overall size of the arrangement. Additionally a small volume of the resonator could yield a fast sequence of consecutive independent measurements (time response). As a compromise we chose the length of 150 mm resulting in a resonance frequency of 1010 Hz for air at STP conditions and a time response of 15 s at a typical flow of 5 L/h.

Since absorption of (scattered) laser radiation can periodically heat up the resonator wall, we tested four resonators, each of a different material or with a different surface treatment, for their suitability to yield a low background signal. Material and surface quality for the resonators are listed in Table 2. To investigate experimentally the influence of the material properties we directed a 1-W cw CO\(_2\) waveguide laser beam at 943.34 cm\(^{-1}\) to the center of the resonators at a grazing incidence angle of 3.2 deg. Expressed in terms of equivalent gas absorption amplitude the PA signal arising from wall absorption can be found in Table 2. The unpolished gold-coated copper tube produced a threefold higher signal than the polished version.

The relatively low absorption amplitude of the polished gold-coated copper resonator, compared with those made from stainless steel and brass, can be understood by comparing the bulk properties of the metals. PA signal generation is proportional to \((\kappa c_p)^{-1/2}\) with \(\kappa\) as the thermal conductivity and \(c_p\), the heat capacity of the bulk material. For stainless steel, brass, and copper this resulted in a ratio of, respectively, 6:2:1. This ratio was approximately observed. The best performance was obtained by employing a copper tube as resonator material with a polished gold coating. Because of the excellent heat-conducting properties the absorbed heat can be quickly dispersed in the copper tube. The gold coating served not only to optimize

<table>
<thead>
<tr>
<th>Material</th>
<th>Experimental</th>
<th>Bulk Properties ((\kappa c_p)^{-1/2})</th>
</tr>
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<tbody>
<tr>
<td>Polished stainless steel</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Polished brass</td>
<td>2.5</td>
<td>2</td>
</tr>
<tr>
<td>Polished gold-coated copper</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unpolished gold-coated copper</td>
<td>3.5</td>
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\(^{a}\)We obtained the results by monitoring the PA signal generated by an amplitude-modulated CO\(_2\) waveguide laser beam directed at the center of the resonator at grazing incidence. Values are scaled to the results of the polished gold-coated resonator.
the reflection of laser radiation, but also to obtain a noncorrosive surface to withstand aggressive gases.

Another source of acoustic background signal resulted from radiation absorbed by the ZnSe window material. Two measures were employed to minimize this background signal (Fig. 1). The first was the use of large buffer volumes between the resonator and the window. Since the trace gas flow was introduced directly to the center of the resonator this hardly affected the time response of the detector. The second was the introduction of tunable air columns close to the windows as described previously.22,23

The performance of the cell was tested with an extracavity CO2 waveguide laser beam modulated by a chopper. The laser beam passed through the first, transparent, ZnSe Brewster window and was partially absorbed in the slightly blackened second window. By tuning the air column at this window we observed a clear maximum at 60-mm and a minimum at 80-mm column lengths in the PA window signal. The window signal could even be reduced by a factor of 40 compared with the situation when there was no air column. A theoretical model has been developed to describe this acoustic behavior.22,23 The results of this model predicted a reduction factor of 50 for the window signal amplitude at a column length of 80 mm. Placing the PA cell into the laser cavity with optimally transparent windows, we observed hardly any effect on the low background signal amplitude by changing the column length. Apparently, the large buffers sufficed to minimize the window signal.

The acoustic signal originating from absorption was monitored by a Bruel & Kjaer (B&K) 4179 condenser microphone. The membrane of the microphone and the volume in front of it were part of the acoustic resonance, thereby influencing the resonance frequency, cell constant, and quality factor.22,23 The use of a small electret Knowles EK 3024 microphone did not show a measurable influence. The above-mentioned theoretical model was applied to clarify this effect. The results corresponded well with the experimental data and could explain the downshift in resonance frequency by 65 Hz. The B&K microphone was advantageous because we used its high signal-to-noise ratio to lower the detection limit.

The lowest nonelectronic noise level can be determined by the Brownian motion of the molecules and poses a limit to the sensitivity of this detector. Although the cell was shielded and mounted to the laser frame on vibrational dampers we did not reach the theoretical limit (0.2 µPa Hz−1/2) because acoustic noise (2 µPa Hz−1/2) was picked up at the resonance frequency, which limited the sensitivity of the system. We therefore could not yet utilize the full potential of the B&K microphone. In the case of trace gas detection of methane in N2 this pickup noise restricted the practical detection limit to 1 ppbv (absorption coefficient α = 3 atm−1 cm−1 at 1304.97 cm−1, 1-W laser power, see Fig. 3).
injectionsof1-mLN 2,containing2.6%ofwatervapor,
under all the peaks is equal.

Figure4

kinetic cooling can hardly take place because of the
strongest water vapor absorption lines, e.g.,
overload of the detector we did not use one of the
mally performed in dry air conditions. Our results
frominsectstobemeasuredtheexperimentsarenor-
conditions. However, to allow the water vapor release
phase lag. It is therefore preferable to measure the
methane absorption substantially and reduced the
flow we increased the PA signal amplitude from
laxation and, indeed, by increasing the humidity of

cuvette.

PAsignal. Thelargebackgroundiscausedbyacockroachinthe

injections on the high background result in a larger
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injections of equal amounts
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Water vapor has a strong absorption band (ν2 at
2330 cm⁻¹), kinetic cooling can hardly take place because of the
large energy gap with respect to the excited vibrational mode of CH4.

Water vapor is known to promote vibrational relaxation and, indeed, by increasing the humidity of the flow we increased the PA signal amplitude from methane absorption substantially and reduced the phase lag. It is therefore preferable to measure the methane release from insects at high humidity conditions. However, to allow the water vapor release from insects to be measured the experiments are normally performed in dry air conditions. Our results did not allow us to determine whether water vapor promoted relaxation of CH4 or O2.

Ethylene and ethane were also tested, but we observed no change of their trace gas signal by introducing extra water vapor into the flow. The introduction of water vapor into the flow yields only a slightly higher background signal arising from water absorption (α ~ 10⁻³ atm⁻¹ cm⁻¹) at the P(10)_{32} CO laser line that was used for CH4 detection.

5. Water Vapor Detection in the Presence of Oxygen

Water vapor has a strong absorption band (ν2 at 1594.7 cm⁻¹) in the CO laser region. To prevent overload of the detector we did not use one of the strongest water vapor absorption lines, e.g., P(12)_{13} (1761.69 cm⁻¹, α ≈ 2 atm⁻¹ cm⁻¹), but a moderate absorbing line P(11)_{19} (1616.04 cm⁻¹, α ≈ 0.3 atm⁻¹ cm⁻¹). In the case of air as a carrier gas a quadratic dependence on the H2O concentration was found. Figure 4(a) shows the PA signal as a result of several injections of 1-mL N2_, containing 2.6% of water vapor, into the carrier flow. When the injections (ΔP) were performed on a large PA water vapor background signal (P) the total PA signal (S) was much larger compared with injections on a low water vapor background signal. Taking the square root resulted in equally sized peaks as shown in Fig. 4(b). Although water vapor is known to be a fast relaxing agent, O2 apparently acted again as buffer for vibrational energy storage that could then be released by collisions with water vapor molecules yielding a nonlinear concentration dependence. Fast relaxation of O2 is to be expected at high water vapor concentrations; in this case the PA signal is linear with the concentration. Further experiments have to be performed in order to elucidate this problem.

Because of the strong adsorption of water molecules to wall material and because of its spectral interference with other gases, outgassing of water vapor from walls could perturb the performance of the detector. We reduced the outgassing problem by inserting a cooling trap and perfluoroalkoxy (PFA) Teflon tubing into the gas-handling system between the sampling cell and the PA cell. In this way we were able to lower the water background signal below 1 ppmv. A detection limit of approximately 100 ppbv was found. Note that a detection limit of 0.1 ppbv in N2, was extrapolated based on noise consideration, absorption coefficient, laser power, and assumed absence of interfering gases. The 100-ppbv detection limit was sufficient to measure the water vapor release from small insects.

6. Performance for Biological Measurements

The dimensions of the buffers of the PA cell (100-mm diameter, 100-mm length) resulted in a total cell volume of 1.5 L. Since we introduced the trace gas flow at the center of the resonator the time response of the detector depended mainly on the volume of the resonator (26 mL). The small diameter gas inlet (0.8 mm) that we used did not influence the cell constant. Acoustic outside noise was effectively suppressed by acoustic notched filters in line with the flow tubes. A notched filter consisted of a small diameter (0.8-mm) and a large diameter (8-mm) tube both of a quarter acoustic wavelength. The noise reduction of such a filter was proportional to the ratio of the cross-sectional areas. The noise reduction by one filter yielded an attenuation factor of 100. During our experiments we used two of these filters, although one filter would have been sufficient.

We tested the properties of PFA Teflon for water memory effects by injecting water vapor into a 5-L/h flow of air and comparing this with injections of methane. The detector had the same time response (15 s) for both trace gases. By switching from a calibrated high concentration trace gas mixture (1.2-ppmv C2H4 in N2) to a pure N2 flow (1 L/h) we observed 15% of the initial trace gas signal after the first hour. This can be explained by backdiffusion from the buffer volumes into the resonator. After having passed through the resonator the residence time of the trace gas in the buffers was typically 1.5 h. Because of backdiffusion former fillings contribute to the actual
volumes, however, could be reduced by a factor of 5. Memory effects from trace gas dwelling in the buffer by backdiffusion would still take place; the problem of L buffers could be purged by additional gas flows of 3 sources with a low carrier gas flow, the gas in the measurements on biological samples.

A minimal flow rate of 1 L at high flow velocities and reduces the concentration. Biologically source, the production rate becomes diluted however, not always desirable since, in the case of a significantly by increasing the flow velocity. This is, of 6 L of 1 cm s~1 for N2.

We performed an extracavity experiment employing the above-mentioned CO2 waveguide laser arrangement to elucidate this problem. We flushed the buffers of the PA cell with N2 and varied the trace gas flow velocity. Under these conditions backdiffusion of pure N2 diminishes the PA signals that otherwise would be independent of the flow rate. The influence of the flow rate on the signal amplitude is shown in Fig. 5. The theoretical cell response curve was simulated by the aid of a computer model that combined the individual absorption contributions of parts of the resonator tube to a total pressure amplitude at the site of the microphone. This approach yields a good description of this undesired effect. At a flow rate of 0.5 L/h the direct trace gas signal yields only 70% of the PA signal at a flow rate of 6 L/h. Thus, backdiffusion could be reduced significantly by increasing the flow velocity. This is, however, not always desirable since, in the case of a biological source, the production rate becomes diluted at high flow velocities and reduces the concentration. A minimal flow rate of 1 L/h was used for the measurements on biological samples.

To allow for measurements from low production sources with a low carrier gas flow, the gas in the buffers could be purged by additional gas flows of 3 L/h through each buffer. Note that signal reduction by backdiffusion would still take place; the problem of memory effects from trace gas dwelling in the buffer volumes, however, could be reduced by a factor of 5.

signal and cause fairly constant background signal decay on a time scale of hours. One can describe this effect by relating the trace gas flow velocity (v = 0.02 cm s~1) to the average distance (d) of backdiffusion into the resonator at stationary conditions, d = Dv/2 (diffusion constant D = 0.185 cm2 s~1 for N2).

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To balance the buffer in and out flow with the trace gas flow we had to monitor the flow rates carefully so that serious problems were not introduced into the system. When we performed the measurements on insects we used trace gas flows of 1 and 5 L/h in the resonator and 3 L/h to purge the buffers. The sample gas flowed over the insect as it entered and left the cuvette by way of capillaries. The animals were kept at ambient temperature (22 °C) inside the glass cuvette (20-mm diameter, 50-mm length), which was sealed at both ends with butyl rubber stoppers. We constructed a cooling trap to remove water vapor from the carrier gas. Thus a dry air flow into the sampling cuvette was obtained for determination of the water vapor release; we positioned the trap behind the sampling cuvette to monitor the release of CH4 by the animal. The cooling trap consisted of trapping stages and a reservoir in which the liquid-nitrogen level was kept constant. Close to the bottom, just above the liquid-nitrogen level of the Dewar, we maintained a metal plate at a constant temperature of 125 K. Near the top we observed a freezing stage of 255 K. The trace gas containing water vapor was first guided through a relatively wide PFA tube (11-mm diameter, 60-mm length) connected to a 255-K plate. At this point most of the water vapor was frozen and, since the tube was easily accessible, we were able to defrost it every day to prevent blockage. For further reduction of the water vapor concentration the flow was guided through a 1-m long (1.6-mm diameter) tube connected to a 125-K plate. Inlet and outlet tubes of the cooling trap consisted of PFA with 0.8-mm diameter (2-m total length).

We performed measurements of CH4 by periodically switching between two laser lines, one at a strong and the other at a weak methane absorbing line. We used the P(9)22 (α = 0.02 atm~1 cm~1) and the P(10)22 (α = 3 atm~1 cm~1) transitions of CO at 1308.01 and 1304.97 cm~1 (see also Fig. 3). We accomplished fast response measurements by keeping the laser at the strong absorption line without switching. We also performed fast water vapor measurements at one single laser line P(11)19 (α = 0.3 atm~1 cm~1). Because of the nonlinear dependence of the PA signal on the water vapor concentration the calibration needed special care. At the start and the end of each biological measurement we used injections of saturated water vapor to calibrate on top of the signal emitted by the animal (see, e.g., Fig. 4).

The fast time response measurements included the determination of CO2 release. The gas flow was thus split; one half was guided to a conventional CO2 infrared gas analyzer (ultrarot absorption 2T, Hartmann & Braun, Frankfurt, Germany) and the other to a PA cell. After installation we synchronized the detectors utilizing the injection of a CO2/CH4 or CO2/H2O mixture into the sampling cuvette. When we adjusted the flow resistances at the outlet of the detectors we obtained equal time responses (and delays) of the injected amount. One can calibrate the detec-

![Image](https://example.com/image.png)

Fig. 5. Comparison between the experimental (filled squares) and theoretical (curve) PA signals generated in the resonator as a function of the trace gas flow (1-ppmv ethylene in nitrogen and resonant CO2 laser transition at 949.749 cm~1). The buffers are flushed with pure nitrogen. At a low flow rate the undiluted nitrogen diffuses back into the resonator, decreasing the effective absorption length. At a high flow rate the cell constant is equal to the value obtained when the whole cell is filled with trace gas.
tors by injection of a specified amount of CH₄, H₂O, or CO₂.

7. Gas Exchange Measurements of Insects

Some 300 million years ago, long before land-dwelling vertebrates evolved, insects appeared on Earth. These have been estimated to consist of more than two million species and are known to constitute an enormous biomass worldwide. Insects and millipedes contribute to the global change of the atmosphere. Evidently, they all release CO₂ and H₂O and consume O₂ as a natural consequence of their metabolism and breathing. In addition, however, millipedes and at least three higher taxa of insects, termites, cockroaches, and scarab beetles, emit CH₄ as one of the end products of their intestinal (bacteria-aided) fermentations. Estimates show that nowadays total arthropod emission could constitute as much as 25% of the mondial methane production. Our initial use of the described detector was to monitor the emission of methane and water vapor from cockroaches and scarab beetles.

In the anaerobic hindgut of CH₄-producing insects, large numbers of bacteria and symbiotic protozoa assist the digestion of biopolymers such as cellulose or hemicellulose. These metabolic activities are fermentations; they take place under anaerobic conditions and yield CO₂ and H₂ as the main products of biopolymer catabolism, to be converted to CH₄ by methanogenic bacteria that also live in the hindgut of their hosts, either free around the partially digested food particles or as intracellular symbionts of intestinal protozoa. In some instances, the insect forms chitinous structures as immobilized supports for intestinal bacteria.

Gas chromatography permits a quantitative cumulative determination of gas emissions; because of a lack of time resolution, no answer could be given to the question of how the intestinal gases are released. Measuring CH₄ simultaneously with CO₂ enabled us to discriminate between a CH₄ release as flatus or as a breathing product.

Insects such as vertebrates consume O₂ and release CO₂ during respiration. However, insects do not possess lungs; they use a tracheal system for gas exchange. This tracheal system consists of a rami- fying system of tubes that connect the internal tissues by way of spiral valves to the atmosphere (see Fig. 6). The spiral valves are controlled by muscles through which the ventral nervous system can isolate the tracheal system from the atmosphere. Similar to other cockroaches, Periplaneta americana possesses ten pairs of spiral valves. Gas exchange between the tracheal system and the atmosphere can take place by active ventilation and by diffusion (no movement). The widespread opinion that insects at rest depend solely on diffusion for gas exchange is based on a model postulated by Krogs and investigated by Hazelhoff. It had been assumed that cockroaches ventilate only during motoric activity; at rest, it was deemed that they did not ventilate at all but exchanged gases by diffusion. However with the aid of diaferometric techniques a cyclic CO₂ release and O₂ intake were also observed in resting insects. Insects released CO₂ in bursts whereas O₂ was absorbed at an almost constant rate. This animals developed a complex breathing pattern presumably to retain their body water vapor. This idea was verified by the observation of Kestler that some insects at rest actively ventilate to exchange gases. During the breathing cycles of insects, three periods can be distinguished that are described briefly. During the constriction phase (C) the spiracle valves are closed; no significant tracheal gas exchange with the atmosphere takes place. The oxygen present in the tracheal system is consumed. The solubility of CO₂ in water is 20-fold higher than that of O₂. CO₂ produced by insect metabolism easily dissolves in the hemolymph that surrounds the tissues. The hemolymph fluid is like the blood for mammals with the exception that there is no major pumping mechanism. Because of oxygen consumption the pressure in the tracheal system drops below the atmospheric value. When the O₂ concentration reaches the lower limit O₂ must be absorbed from the outside.

During the second period (fluttering F) the spiracles open for only a short time allowing a convective influx of air in accordance with tracheal gas under pressure. On the average O₂ is taken in and consumed at a constant rate. Only minor amounts of CO₂ are released, since it has to diffuse against the infrushing air. Therefore, the CO₂ concentration in the hemolymph continues to increase.
At a specific moment an increase of the exchange of tracheal gases with the atmosphere can be observed. When the spiracle valves are open, the gas exchange takes place by active ventilation (V) because of the movements of the abdomen (the posterior part of the insect). One active pumping volley consists of a rapid expiratory stroke, a maintained compression, and then inhalation. This constriction–fluttering–ventilation (CFV) cycle provides the cockroach with the necessary O₂ and allows the metabolic CO₂ to escape to the atmosphere. Note that in parallel gas exchange takes place by the cuticula (the skin); in particular, this leads to water loss and CO₂ release.

8. Biological Results

Figure 7(a) shows a characteristic methane release pattern of a Periplaneta americana cockroach. During this experiment we switched the grating of the laser between two positions; the time resolution was, therefore, only 2 min. The background signal was constant during the course of the measurement, so that the difference signal was due to methane. Nearly five complete cycles are displayed. Within one cycle three periods can be distinguished; a minimum release (C) close to the baseline that lasts for approximately 3 min, followed for 20 min by a slowly increasing signal that levels off (F), and a burst of methane over a period of 6 min (V).

For another Periplaneta americana cockroach simultaneous PA measurements of methane on one laser line and of CO₂ with an infrared gas analyzer are shown in Fig. 7(b). The level of CH₄ release is 1 order of magnitude higher than for the cockroach in Fig. 7(a). These differences of 1 order of magnitude were frequently observed for single cockroaches. In Fig. 7(b) the cycles of methane and CO₂ releases took less time (17 min). Both CH₄ and CO₂ exhibit all the characteristics of a CFV breathing pattern. The minimum corresponds to C, followed by an increased release rate denoted by F. The burst V signifies the period during which the cockroach ventilates. Release of CH₄ and CO₂ shows synchronous but slightly different patterns. The onset of V is somewhat delayed for CO₂. The ratio of releases during F and V is much larger for CO₂ than for CH₄. CO₂ emission shows a gradual decrease toward the next constriction period. In contrast, methane stays at a constant level until the start of C. Note that no corrections were applied for the background contribution.

Figure 7(c) shows water vapor release by a third Periplaneta americana. The water vapor signal shows an offset that is due to continuous release from the animal, probably caused by cuticular losses. Because of the calibration problems with water vapor the scale gives only an indication of water vapor. A possible scale of a factor of 1.5 seems realistic. Released patterns of CO₂ and H₂O that are the result of breathing are almost identical. The changing baseline for the CO₂ released pattern is the result of a drift of the signal in the infrared gas analyzer.

Fig. 7. CH₄, H₂O, and CO₂ release patterns of the Periplaneta americana cockroach (40 mm long, fresh weight 1 g). (a) CH₄ release was measured by switching between two adjacent laser lines, one strongly [P(10)₂] and one weakly [P(9)₂] absorbing line. Time resolution was limited by the switching (2 min) and not by the flow in the PA cell (1 L/h). (b) The total flow rate (5 L/h) over the animal was split into two parts, one entered the infrared gas analyzer (4 L/h) for CO₂ detection; the other (1 L/h) entered the PA cell for CH₄ monitoring. Both gases were measured with an increased time resolution of 15 s since CH₄ was determined at only one laser line [P(10)₂]. The synchronous observation indicates that methane was released during the breathing of the animal. The phase shift is connected to the higher solubility of CO₂ in the hemolymph. (c) H₂O and CO₂ were measured simultaneously at a total flow over the animal of 10 L/h (split into two equal parts).
Figure 8 shows the release of CO$_2$ and water vapor by a giant (6-g) *Gromphadorhina portentosa* cockroach. The same characteristic CFV cycle can be observed as for *Periplaneta americana* but during (V) single pumping volleys are resolved as peaks caused by active ventilation. The full width at half-maximum of these peaks corresponds to the time response of the detector (15 s). The individual cycles last for approximately 1 h; all the features are synchronous for the release of CO$_2$ and H$_2$O.

A different release pattern was found for the *Pachnoda bhutana* scarab beetle for which methane and CO$_2$ releases were observed to occur synchronously [Fig. 9(a)]. Every 1.5 h a large amount of CO$_2$ and CH$_4$ was released in a single burst that lasted for approximately 6 min [see Fig. 9(b)]. A CH$_4$ burst lasted for a shorter period of time than did a CO$_2$ burst. Figure 9(c) displays the water vapor release in combination with CO$_2$ from another scarab beetle. After the start of the measurement the water vapor background release showed a slowly decaying release rate. Except for the larger background signal the water vapor release revealed a pattern similar to that...
of CH$_4$ and CO$_2$. Expansion of the horizontal scale shows, however, that in contrast to CO$_2$ and ethane the water vapor release was almost constant during a burst.

All the release patterns presented in Figs. 7–9 show that CH$_4$, CO$_2$, and H$_2$O were emitted synchronously. Therefore, the cyclic release of CH$_4$, in particular, can be attributed to tracheal gas exchange, which proves that methane is not emitted by flatulence. Cyclic water vapor release was observed on top of a fairly constant background signal that has been attributed to cuticular water losses.

Gas exchange that is due to ventilation is accompanied by exhalation of gas. The time response of the system (15 s) allowed us to identify only the gas release of individual pumping volleys in the release pattern of Periplaneta americana. The peaks of gas release from Gromphadorhina are better resolved and demonstrate clearly that this animal ventilates for its gas exchange.

For Pachnoda bhutana the slow decay in water vapor background release is caused by the change of humid ambient to arid conditions in the cuvette at the start of the measurement. The exterior parts of the animal still retain a relatively high amount of water that is slowly released under the arid conditions in the cuvette. The burst period of the gas release does not seem to allow for ventilation and gas exchange seems to be diffusive. The different release patterns for the three gases during the burst cannot be explained solely by their different diffusion coefficients. The CH$_4$ molecules and water vapor possess equal mass and show similar release patterns whereas CO$_2$ (since it is heavier) yields a slower decay. Other mechanisms must be involved to explain the different release patterns.

The almost constant water vapor release during the burst suggests that the beetle keeps its valves open constantly and that inside the tracheal system there is a fairly constant partial pressure of H$_2$O close to the saturation pressure. The slow decrease of CO$_2$ release during the burst is caused by continuous emission of the CO$_2$ dissolved in the hemolymph. In contrast, the release of CH$_4$, practically undisolved in the hemolymph, occurs 1 order of magnitude faster. For methane, refilling the tracheal system occurs less efficiently than for CO$_2$ because of the order of magnitude difference in solubility.

9. Conclusions

An intracavity photoacoustic cell inside the resonator of a liquid-nitrogen-cooled CO laser was employed to measure trace gas release (H$_2$O and CH$_4$). By differential trapping of possibly interfering water vapor (for CH$_4$ at 125 K, after which the major part of H$_2$O was removed at 255 K) we detected methane essentially as a one-component-in-air detection. For fast measurements we used laser radiation from a single laser transition whereas for more precise determination we used the difference between two laser wavelengths for which the sampling time increased from 15 s to 2 min. The PA cell was especially designed to minimize wall effects and window signals. Water showed a nonlinear dependence on concentration, a surprising result traced back to kinetic cooling. Oxygen molecules with their high number density and their slow relaxation act as a vibrational energy buffer and transfer the energy into translational energy by collisions preferentially with water molecules.

The CH$_4$ signal also appeared to be strongly influenced by the presence of traces of H$_2$O. Again, oxygen played a detrimental role as an energy buffer. Detection limits of 10 ppbv in dry air and 1 ppbv in nitrogen were obtained. For water vapor the detection was limited by the amount of background H$_2$O vapor present in the cell allowing a detection limit of approximately 100 ppbv. Measurements of living insects revealed that the time response (15 s) of the instrument was sufficient to detect characteristic breathing patterns of cockroaches and scarab beetles. For the first time, to our knowledge, methane was found to be released through breathing.

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References