Laser Photothermal Deflection applied to Local Trace Gas Detection; Respiration of Tomatoes

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Abstract
A laser-driven trace gas monitor is presented based on the mirage effect. In an intracavity CO₂ pump laser setup trace gases are vibrationally excited. Due to collisions the well defined excitation region is locally warmed up. A HeNe laser probes the ensuing decrease of refractive index and becomes deflected. The sensitivity of the system is augmented by a multipass configuration for the probe beam to detect ambient ethylene and ammonia concentrations of 0.5 ppbv and 0.25 ppbv, respectively.

Introduction
The photothermal deflection (PD) technique, based on a thermally induced change of refractive index (mirage effect), has been applied in many fields of research (Bicanic, 1992). Here a CO₂ laser-driven PD instrument is described for in situ trace gas detection especially of ethylene and ammonia at sub-ppbv levels (1×10⁹ molecules); biological results will be discussed.

PD is related to photoacoustics (PA). Both methods are based on collisional relaxation of vibrationally excited molecules, resulting in a temperature rise. With PA the temperature increase is probed by microphones via the associated pressure change, see the preceding paper. PD makes use of a gradient in refractive index caused by local temperature increase. A light beam passing over the heated region is deflected and its displacement is measured with a position sensitive detector (de Vries et al., 1994). In case of PD trace gas concentrations can be determined locally in open air, thus avoiding wall adsorption problems (e.g. for ammonia) and changes of reactive gas concentrations (e.g. for ozone).

Special attention is paid to practical instrumentation to obtain high sensitivity and high resolution in space and time. The thermally induced change of refractive index is produced inside the cavity of the CO₂ laser, where the power amounts up to 100 W. Air turbulences are minimized by the introduction of a double probe beam setup. The absorption of ambient (high) levels of CO₂ is suppressed by the use of a sealed-off CO₂ laser. High spatial resolution is accomplished by use of appropriate mirrors in the multiple pass system of the probe laser. The time resolution is enhanced by an oscillating prism employed as line-selective-element for the CO₂ laser.

We present the photothermal technique as a useful tool for the local detection of ethylene emission from intact cherry tomatoes. In general, ethylene acts as a gaseous hormone, involved in the abscission of leaves, germination of seeds, growth and senescence of plants, the wilting of flowers and wounding effects; respiration and ethylene production go hand in hand during the ripening of climacteric fruit (Abeles et al., 1992).

Technical details
The PD setup consists of two frames (fig. 1); one frame for the CO₂ laser and the other frame for the probe laser, its multipass mirrors and position sensitive detector. The latter frame is rigidly fixed to the first one supporting the cavity of the CO₂ pump laser.

The gas mixture inside a quartz waveguide tube (inner diameter 3 mm, length 400 mm) is continuously replenished at a pressure of 60 mbar. It consists of He, CO₂ and N₂, in a ratio of 65 %: 13 %: 22 %. In the waveguide tube a high voltage de-discharge generates radiation between 9 and 11 μm, for about 90 laser lines. At one end of the waveguide tube a grating is positioned as line selective element; a convex ZnSe lens (f=75 mm) and a concave ZnSe outcoupling mirror (R=270 mm) are placed at the opposite side. The distance between lens and mirror (i.e. the available space for investigated samples) is 350 mm.

The cavity length, from grating to outcoupling mirror, is 1.2 m. The outcoupling mirror has a reflectivity of 98.4 %, yielding a typical intracavity...
power of 100 Watt on the strongest lines. Due to the concave output mirror and the lens (f=75 mm) the laser beam possesses a sharp intracavity focus with a waist of 0.28 mm.

The CO₂ laser can be replaced by a sealed-off ¹³C₀₂ laser, thus minimizing the absorption of ambient CO₂. The wavelength region is shifted by about 50 cm⁻¹ to the red as compared to a ¹²C₀₂ laser. Fortunately, ammonia possesses several nearly resonant absorption lines for this laser, while CO₂ absorption is reduced by one order of magnitude.

The gas mixture contains He,¹³C₀₂, N₂ and Xe (5:1:1.44:0.23) at a pressure of 50 mbar. In the discharge tube some ¹³C₀₂ is dissociated into ¹³CO molecules and O₂ and oxides of nitrogen. This dissociation rate depends on the current density and residence time of the gas in the discharge tube (0.15 s; gas flow of 60 l/h; discharge volume of 2.8 ml). Xe is added to decrease the current density, from typically 2.5 mA · mm⁻² to 1 mA · mm⁻², without reduction of laser power. In our sealed-off setup a closed circulating gas flow is provided based on convection without active pumping. Convection is induced by elevated temperatures in the discharge, while the gas mixture in the buffer volume assumes room temperature. Oxidized nickel electrodes act as catalysts re-converting ¹³CO and O₂ to ¹³C₀₂ (Damitras et al., 1976).

The sealed-off ¹³C₀₂ laser (discharge length 400 mm and an out-coupling mirror with a reflectivity of 90%) has been working with constant output power of 3 Watt for at least 200 hours on the strongest lines in the 10R and 10P branches. This amounts to an intracavity power of 35 Watt.

Our practical solution to measure a specific trace gas absorption is based on rapidly switching between two laser lines, achieved by installation of a ZnSe prism (Laser Power Optics, USA), mounted on a galvo element between grating and discharge tube. The prism is similar to a Brewster window of 25.4 mm; however, one side is inclined with respect to the other by 2°. The prism is installed at Brewster angle minimizing power losses to 18% on the strongest laser line.

Employing a flat mirror system the HeNe probe laser passes over the heated region several times. Our multipass system consists of two square flat mirrors (flatness λ/20) with highly reflective ‘Maxbrite’ coatings (R=99.5 %), tilted with respect to each other by a small angle θ. In our actual experiments the number of passes is 31 with θ=1° and a mirror distance of about 40 mm.

To increase the spatial resolution two cylindrical mirrors are used, alternatively. The cylindrical mirrors have a focal length of 45 mm and are also coated with a Maxbrite layer. The incoming probe beam is focused by a cylindrical lens (f=76.2 mm; V-coating) near to the center of the mirror setup with an offset of 1 mm; twice the offset yields the spatial resolution. The maximum number of passes has been 15. The incoming beam is made parallel by a second identical lens. As compared to the flat mirror setup the probing length is decreased from 25 mm to 2 mm length along the CO₂ laser; the vertical resolution of 0.05 mm remains unchanged.

The deflection of the probe laser beam is measured with a position sensitive detector, consisting of a diode quadrant detector with a voltage slope of 0.5 V/mm between two quadrant parts. The resulting signal is fed into a lock-in amplifier. The HeNe probe beam is divided into two parallel beams by a beam splitter. The vertical distance between the two beams is kept small; in this way air turbulence influence both beams in rather the same way. One of these beams passes directly over the CO₂ laser beam and is deflected due to the mirage effect; the other beam passes at a distance of 7 mm from the CO₂ laser beam and is unaffected by the temperature profile. This probe beam serves as reference. The geometrical configuration of both beams is similar in all aspects. Each beam is detected by an individual quadrant detector. The detected signals are subtracted in a lock-in amplifier. In the actual setup, the signal-to-noise ratio is improved by a factor of two under our noisy lab conditions.
Fig. 4  PAD measurements of ethylene emission rates of an orange cherry tomato under initially aerobic conditions. After 2.3 hours an anoxic period was started and at t=5.2 h the aerobic conditions were restored. The dotted line indicates undisturbed aerobic ethylene emission; the dashed line represents the presumed ethylene production if the effect of the conversion of accumulated ACC to ethylene is neglected.

**Respiration and gas emission of tomatoes**

The PD setup is applied to investigate ethylene emission pathways of cherry tomatoes (*Lycopersicon esculentum* 'Cherry'). The tomato is placed under the crossing of the two laser beams, 1.5 mm below the pump laser beam. The measurements are carried out at 21 °C under normal atmospheric conditions, locally and instantaneously.

The intact cherry tomatoes were in the mature orange stage, weight approximately 14 g, and diameter about 35 mm. The tomatoes were detached from the plant and in most cases the coronet (including pedicel and sepals) was removed. The thus treated fruit showed enhanced respiration as testified by higher levels of CO₂ emission and especially by increased emission of ethylene. This behavior is typical for climacteric fruit (Abeles et al., 1992).

Measurements were performed at the stem-scar site (where the coronet had been situated), the bottom site (opposite to the stem-scar area) and an equatorial site. The data are presented in fig. 2. Most of the internally produced ethylene is released at the coronet site of the tomato (90%). The amounts of ethylene emitted at the bottom site and at the equator both are very low; levels are just above ambient, which are of the order of 4 ppbv.

The PA technique discussed in the following contribution by Bijnen et al. offers an alternative route to measure local gas emission, see fig. 3. A double cuvette has been developed where gas emission from the coronet area can be measured simultaneously with the integral emission from the remaining surface of a tomato (Bijnen et al., 1994).

The coronet side forms the nearly exclusive connection to the atmosphere where gas-uptake and -release occur during the respiration processes of tomatoes. Blocking this passage e.g. by parafilm leads to anaerobic conditions in the fruit and onset of fermentation. If after 2 hours the passage is opened again the consequences of lack of oxygen are virtually the same as those shown in fig. 4. The emission of ethylene is re-established slowly; after about 10 hours the emission rate is back to normal, i.e. ACC (1-aminocyclopropane-1-carboxylic acid) as precursor of ethylene is produced as before. There is a significant first peak at about t=2h after reopening of the coronet passage indicating the presence of ACC stored during the anaerobic interlude that is converted to ethylene before renewed ACC production gets started. Note that fig. 4 belongs to a PA measurement where the coronet passage remained unobstructed and where anaerobic conditions were imposed replacing oxygen by nitrogen – for two hours – in the gas flow through the measuring cuvette.

In fig. 5, the response of the ethylene emission from tomatoes is displayed for fast changes between anoxic and normal atmospheric conditions. In this case gas is sampled from the stem-scar region,
using the upper small measuring compartment displayed in fig. 3. Fig. 5 demonstrates on one hand how fast the biosynthesis of ethylene is interrupted upon lack of oxygen and how fast it is resumed; on the other hand, it shows how well the PA technique with its time resolution of 1s is suited to follow fast physiological processes. Note that the PD technique even is at least one order of magnitude faster as has been demonstrated for ammonia measurements in the open atmosphere (de Vries et al., 1994). The physiological processes occurring in fruit during and after the anoxic period seem to be very similar to what takes place in very different plant tissues. In fig. 6 the ethylene emission is shown for 25 germinating peas; also here an anoxic period of about 2 hours first causes the signal to drop to almost zero and then - after re-admission of oxygen - a large peak indicates conversion of stored ACC to ethylene; thereafter normal ACC- and ethylene-production are resumed.

Recent achievements

We want to conclude this contribution with some recent achievements.
- From an environmental point of view deposition- and emission-fluxes of ammonia determine the influence of ammonia on ecosystems. Ammonia is known as an important nitrifier of the soil which can be disastrous for certain ecosystems, due to its toxicity already at levels of 100 ppbv. Concentration measurements at a repetition rate of 10 Hz, at sub-ppbv levels are required for flux determinations by the eddy correlation technique (Slanina, 1993). The combination of the $^{13}CO_2$ laser, the oscillating prism and the multipass mirror system in the PD setup satisfies these requirements.
- In a first attempt PD has been successfully applied to determine ammonia levels above a culture of cyanobacteria in water, after treatment by an inhibitor of nitrogen assimilation.
- Similarly, minute amounts of ammonium in water (i.e. the ammonium-content of water samples) have been measured adding NaOH to these samples and following the ammonia levels above the water surface, in real time.
- The strong influence of about 90 ppbv of ozone on germinating peas has been investigated. PD techniques are important because the concentrations of this reactive trace gas above the peas have been observed to change greatly due to wall effects. Ozone intermittently applied leads among other effects to sharp responses in ethylene emission by peas.

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