Individually ventilated cages microclimate monitoring using photoacoustic spectroscopy

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Abstract:

A multi-gas photoacoustic sensor was developed in order to monitor microclimatic parameters at ppm level from the atmosphere of individually ventilated cages housing mice. Ammonia, water vapour and carbon dioxide were measured on-line and in real-time during two weeks and 24 hours a day. The quality of bedding and the ventilation rate inside the cages has been monitored. The circadian activity level of these animals has then been observed.

1. Introduction

Photoacoustic spectroscopy (PAS) is a spectroscopic technique widely recognized for its high performances in low concentrations measurement. Associated to laser sources, this method becomes very attractive for gas monitoring at trace level, from hundreds of parts-per-million (ppm) to parts-per-billion (ppb), due to its simplicity, high sensitivity and large selectivity. Furthermore, this technique enables on-line and real-time measurement with a short response time and without sampling requirement. It is thus very suitable for continuous gas monitoring in various fields of applications, such as atmospheric pollution measurements, industrial process control or medical applications. In addition, the use of fibre-coupled semi-conductor distributed feedback (DFB) lasers offers an interesting potential for the realisation of multi-species sensors, as the use of optical fibres facilitates the coupling of several lasers into one measurement photoacoustic (PA) cell. We report here an application of a multi-gas PA sensor in life sciences for the continuous recording of microclimatic parameters at ppm level from the atmosphere of individually ventilated cages (IVC) housing laboratory animals such as mice. Microclimate level of different physiological gases such as water vapour (H_2O), carbon dioxide (CO_2) and ammonia (NH_3) is relevant to the well being of the laboratory animals, an important contribution to the quality of *in vivo* research. These species have been simultaneously measured in real-time and at ppm-level with a single instrument based on three near-infrared DFB lasers and a resonant

PA cell. A brief description of PAS is presented and the experimental part with on-site measurement is developed.

2. Basics of photoacoustic spectroscopy

PAS is a calorimetric method, in which the optical energy absorbed in a gas sample is directly measured through the heating produced in the medium. The conversion from optical energy to heat is induced by molecular absorption of photons at proper wavelength and subsequent non-radiative relaxation of the vibrational excited state (collisional relaxation). The small local temperature variation in the sample is associated to a pressure variation. When the deposited optical energy is modulated (for example by an intensity or wavelength modulation of the laser), a periodic heating is produced, thus generating a modulation of the sample pressure. This results in an acoustic wave, which is detected using a miniature microphone. The amplitude S_{PA} of this acoustic wave can be significantly enhanced using an acoustic resonator configuration. In such a case, the PA cell is designed to be acoustically resonant and when the laser modulation frequency is adjusted on a resonance frequency of the cavity, an amplified standing wave is created in the cell. The amplitude of this wave depends linearly on the laser power P_0, the molecular absorption coefficient α and on a parameter characterizing the geometry of the PA cell, the cell constant C_{cell}: $S_{PA}=C_{cell}\ \alpha\ P_0$.

3. Design of the photoacoustic sensor

Our home-made PA cell has been optimised to be operated on its first longitudinal mode [1]. It is built out of stainless steel and consists in two large buffer volumes and a central cylindrical tube of radius R_c = 3 mm acting as an acoustic resonator (see Figure 1). Three fibre-coupled DFB laser diodes emitting at 1369 nm for the detection of H_2O, at 1572 nm for the detection of CO_2 and at 1531 nm for the detection of NH_3 are used. The light from the two first lasers is combined with a 5/95 coupler. The output of the coupler ends with a beam collimator directly mounted on the face of the first buffer volume of the PA cell, which facilitates the light coupling into the cell. A strongly asymmetric coupling ratio is used between the two lasers in order to keep the highest incident power into the PA cell for the detection of CO_2, as the absorption of this molecule in the spectral range of interest is quite low. The resulting loss of power of the laser for water vapour monitoring does not have any consequence, because a detection limit in the 20 ppb range is achievable by our sensor with the full laser power, whereas much higher concentrations (in the

percent range) have to be measured in this application. The fibre from the third laser (NH_3 detection) also ends with a beam collimator that is mounted on the face on the second buffer volume of the PA cell. This configuration enables to benefit from the entire optical power of this laser, thus improving the detection limit of NH_3, which is necessary to detect the low NH_3 concentration encountered in this application.

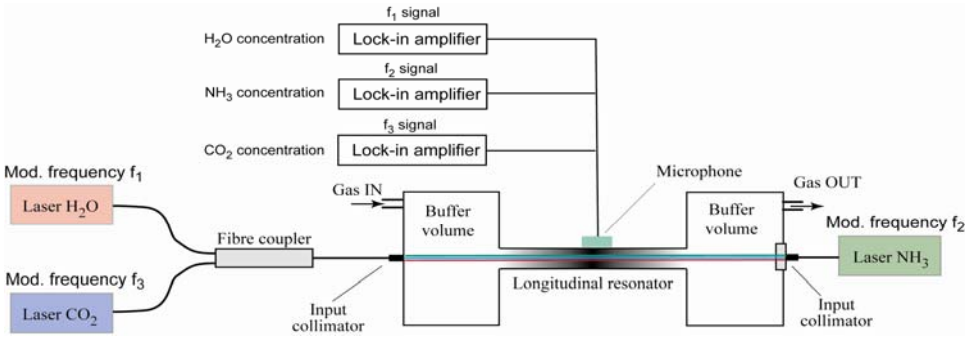

Fig. 1: Schematic representation of the PA sensor based on three DFB lasers and a PA cell operated in its first longitudinal mode. The lasers are modulated at three different frequencies and three lock-in amplifiers extract the corresponding concentrations from the microphone signal.

Each of the buffer volumes of the cell was built with a movable piston, enabling to easily adjust its length and to study its influence on the coupling between the ambient acoustic noise and the resonator. The laser beams go through the cell on its axis in order to efficiently excite the first longitudinal mode of the resonator. The sound waves generated by the three laser beams are detected with the same electret microphone located at the centre of the resonator, i.e. at the maximum of the acoustic standing wave. In order to separate the acoustic signals generated by the different lasers, they are modulated (modulation of their injection current) at three slightly different frequencies, all located in the resonance curve of the cavity (see Figure 2). The laser for NH_3 detection is modulated at frequency f_1, corresponding to the centre of the resonance in order to benefit from the largest acoustic amplification. A quality factor Q=28 is achieved in the resonator. The lasers for H_2O and CO_2 detection are respectively modulated at frequencies f_2=f_1-5 Hz and f_3=f_1+5 Hz. With so small differences between frequencies f_1, f_2 and f_3, the loss in the acoustic amplification is tiny, whereas cross-talk between the generated acoustic signals is suppressed using three lock-in detection at frequencies f_1, f_2 and f_3 respectively. Therefore, this configuration enables the simultaneous and independent measurement of the three species of interest. An electronic module controls the laser modulation and processes the data.

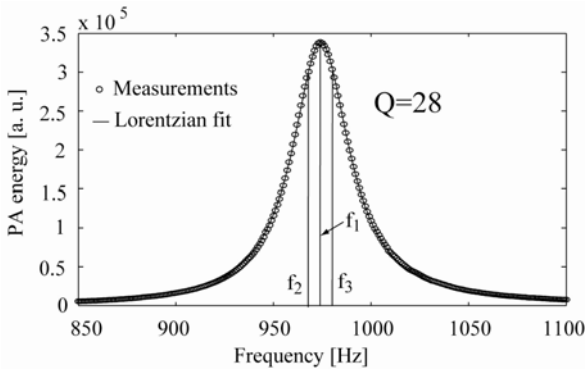

Fig 2: First longitudinal acoustic resonance of the PA cell. Circles are experimental points and the curve is the result of a lorentzian fit. The three laser modulation frequencies f_1, f_2 and f_3 are represented.

4. Experimental results

4.1 Response of the system

PA spectra of water vapour, carbon dioxide and ammonia have been measured by tuning the lasers temperature and by recording the generated PA signals. They are shown in Figure 3 and are compared with the absorption spectra calculated from Hitran database [2], excepted for ammonia for which no data is available in this spectral range. A good agreement is obtained between PA measurements and calculated spectra.

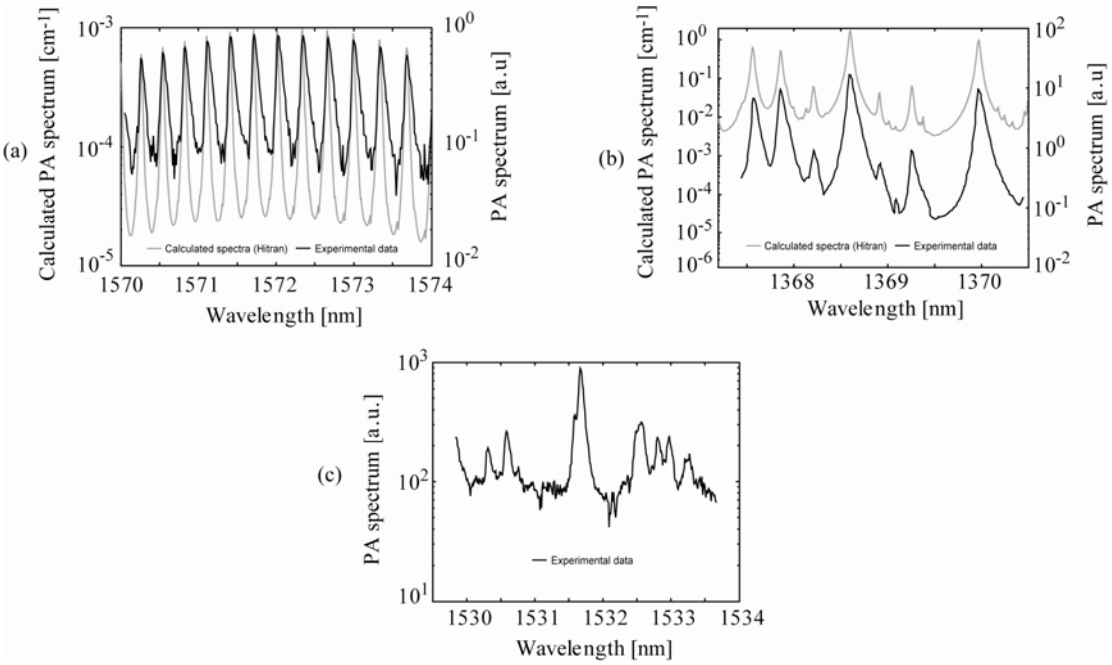

Fig. 3: PA spectra measured with our experimental set-up. Black curves show experimental data and the grey lines represent the corresponding absorption spectrum calculated from HITRAN database. (a) CO_2; (b) H_2O; (c) NH_3.

The response of the system to various ammonia concentrations is shown in Figure 4. The temperature of the laser is tuned to reach the appropriate absorption line and the current modulation amplitude is optimised to achieve the strongest PA signal. Different gas mixtures are obtained from certified concentrations diluted with mass flow controllers. It can be seen that an excellent linearity is obtained. A similar behaviour has been achieved for CO_2 (not shown here), whereas we were in the nonlinear part of the absorption curve for water vapour at the high humidity levels to be measured in this application. The flow rate used for each measurement is 1 l/min which is the maximum before increasing the noise level. Sensitivity for each gas has been determined from the noise level of the sensor. By taking a signal-to-noise ratio *SNR*=3 a sensitivity of 0.2 ppm of ammonia, 40 ppm of carbon dioxide and 0.5 ppm of water vapour has been obtained. The dominant sources of noise are the intrinsic noise of the microphone and the ambient acoustic noise.

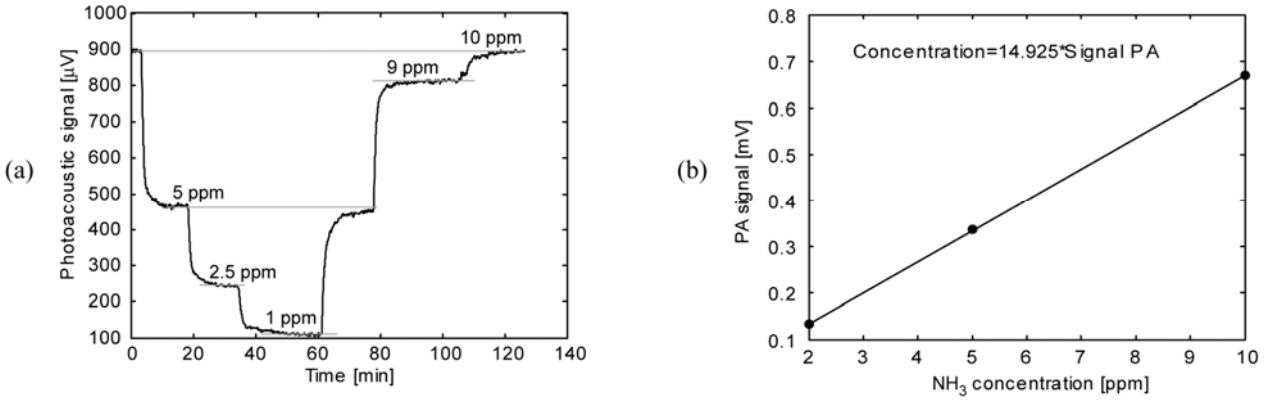

Fig. 4: (a) Response of the system to different NH_3 concentrations; (b) Calibration curve.

4.2 On-site measurements

On-line and real time measurements of ammonia, water vapour and carbon dioxide were performed during a period of two weeks in IVC housing laboratory mice. The first two gases reflect the quality of bedding and the ventilation rate inside the cage and therefore the level of hygiene. Carbon dioxide is an indicator of physical activity of living organisms allowing to assess the circadian activity of the mice in the cages. Fourteen cages containing a different number of animals have been monitored; they are listed in Table 1.

At the output of the cage, air is pumped into a Teflon tube at a flow rate of 1 l/min. The flow passes through the PA cell and the concentration of the three gases is measured. After the cell, the air is injected back into the cage, avoiding additional ventilation that could perturb the microclimate inside the cage (see Figure 5).

Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Cage 7
6F	2F	3F	1M&2F	6M	5F	M&F&11P
Cage 8	**Cage 9**	**Cage 10**	**Cage 11**	**Cage 12**	**Cage 13**	**Cage 14**
5M	1F	M&F&7P	M&F&11P	1M&2F	5F	2M

Table 1: Cages description. M stands for male, F for female and P for pups.

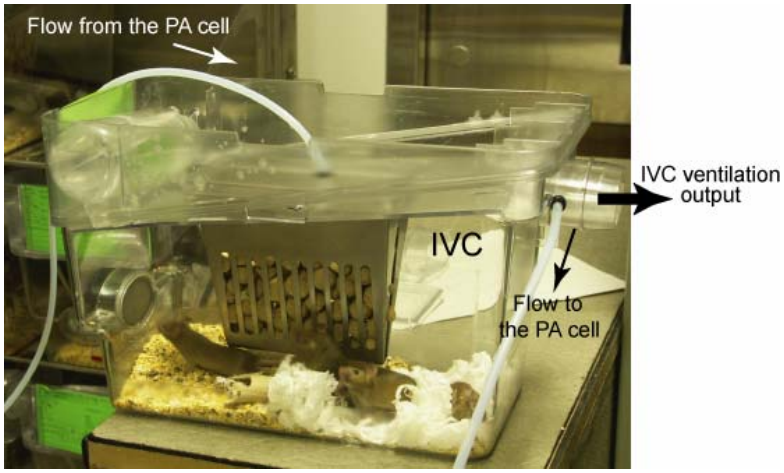

Fig. 5: View of the inlet and outlet of the tubes in an individually ventilated cage.

Ammonia concentration was monitored in the different cages at various days after the bedding cleaning. The result is plotted in Figure 6. After day 7, one cage has a concentration over the limit of 25 ppm, which corresponds to the threshold limit value for chemical substances in the workroom environment for humans [3]. After 11 days (date of the next bedding change), an additional cage presents a value over this threshold and three others are close to it. A high level of ammonia, close to 70 ppm, has been observed in the dirtiest cage.

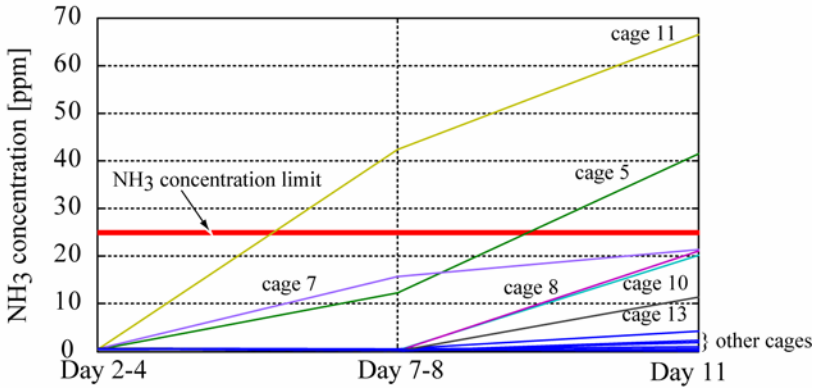

Fig. 6: Evolution of ammonia concentration from day 2 to day 11 in the fourteen cages.

CO_2 and H_2O concentration monitoring during three days is presented in Figure 7. CO_2 variations show a 12-hours time period, representing night and day activity. Correlation between water vapour and carbon dioxide cycles are also observed, showing an increase of perspiration of active animals.

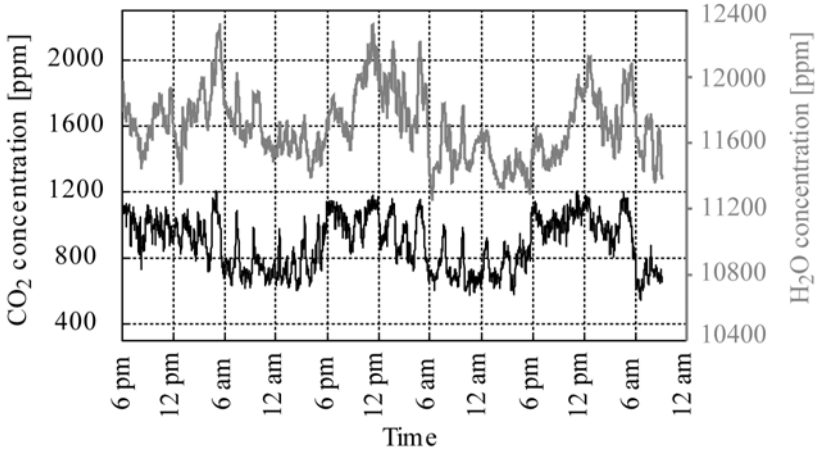

Fig 7: Evolution of H_2O and CO_2 levels in one cage from day 4 to day 7.

CO_2 monitoring is also important as it enables the detection of eventual ventilation breakdowns for which an increase of the CO_2 concentration in the cages may be critical for animal's survival. CO_2 was monitored in cages disconnected from to the ventilation (see Figure 8).

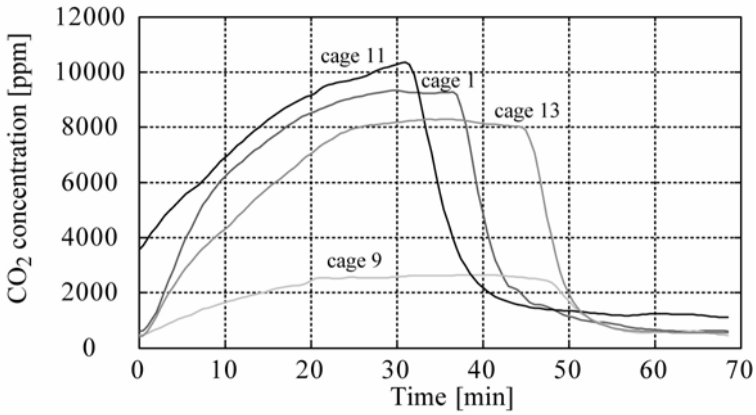

Fig. 8: CO_2 accumulation in cages disconnected from the ventilation. An equilibrium level is reached after 30 to 45 minutes.

A dramatic increase of CO_2 can immediately be observed after the aeration interruption, showing the importance of adequate working ventilation. The air renewal flux of the cages has also been measured by injecting a fixed carbon dioxide concentration (5000 ppm) into an empty cage and measuring the CO_2 concentration decay when the injection is stopped (see Figure 9). The CO_2 measurement shows an exponential decay curve with a time constant of 2 minutes, corresponding to an air flow of 4.86 l/min for a cage of 9.75 litres. Total air change in the cage

(95% of CO_2 washed out) is estimated to be equal to 3 cage volumes. Therefore, a flow of 4.86 l/min corresponds to a total of 10 cage air renewals per hour.

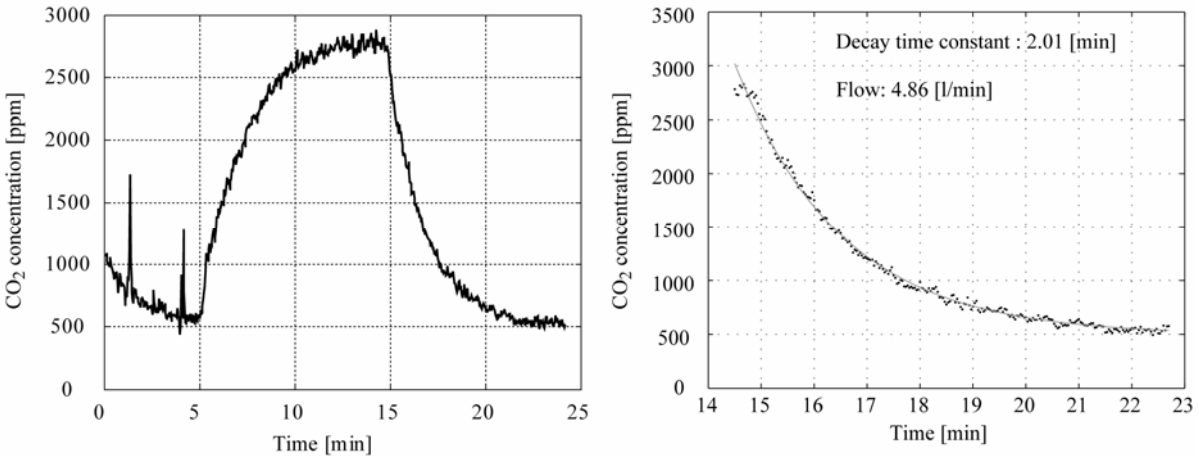

Fig. 9: Measurement of the ventilation flow of an empty cage. A fixed CO_2 concentration is first injected into the cage. When an equilibrium state is reached, the injection is stopped and the decay of the CO_2 concentration is monitored. The flow is obtained by an exponential fit on the experimental data.

5. Conclusion

Monitoring of microclimatic parameters in individually ventilated cages has been performed during two weeks and 24 hours a day using a multi-gas photoacoustic sensor. Simultaneous measurement of carbon dioxide, water vapour and ammonia has provided a better understanding of the evolution of the bedding and the ventilation of the cages. These results fully demonstrate the suitability of the photoacoustic technique for this application, thanks to its high sensitivity and fast response time. Circadian activity level of animals can also be studied with this technique.

[1] Besson J.-Ph., Schilt S., Thévenaz L.: Multi-gas sensing based on photoacoustic spectroscopy using tunable laser diodes. Spectrochim. Acta A 60 (2004), 3449-3456

[2] Dewey C.F., in: Pao Y.-H. (Ed.), Optoacoustic spectroscopy and detection, Academic Press, New-York, 1977, Chap. 3, pp. 47-77

[3] Gamble M.R., Clough G.: Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. Lab Animals 10 (1976), pp. 93 – 104.