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Determination of photochemical loss in leaves by an open-ended photothermal cell

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Abstract

The 'photochemical loss', here designated by L, of an intact leaf of *Nicotiana tabacum* was determined both at low and high frequencies of modulation using an open-ended photothermal cell and a conventional photoacoustic cell. The results confirm a previous supposition that this parameter is frequency independent. We can measure L at low frequencies because the open-ended photothermal cell is insensitive to the oxigen evolution. Working at such low frequencies is advantageous because of the better signal-to-noise ratio compared to that of a conventional photoacoustic cell operated at high frequency. The use of the open-ended photothermal cell has an additional advantage due to the fact that measurements can be made with the leaf still attached to the plant. © 1997 Elsevier Science B.V.

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1. Introduction

With conventional microphone photoacoustic cells it is not possible the determination of the 'photochemical loss' in leaves at low modulation frequencies [1]. This happens because at low frequencies, below 200 Hz [2], the microphone detects a photoacoustic signal which is the vectorial sum of two components; the photothermal and photobaric ones. The photothermal component arises from the conversion of the modulated light in modulated heat which causes an acoustic wave [3]. The photobaric component is due to modulated photosynthetic oxygen evolution [2]. Therefore for the determination of L, which is the ''reduction of the thermal part of the photoacoustic signal by a fraction equal to that part of the photon energy which is stored by the photosynthetic process as chemical energy'' [1], it is necessary to work at high modulation frequencies (e.g., 400 Hz) in order to avoid the modulated oxygen evolution, which is damped out at these high frequencies [1]. On the contrary by using the 'open-ended photothermal cell' [4] it is possible the determination of L at low frequencies since the $LiNbO_3$ crystal transducer used in this cell does not detect the oxygen evolution [5] because of the poor acoustical impedance matching between the gas and the crystal (a similar transducer was developed, with antecedence of 4 years, by

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Tomonobu Hata et al. for spectroscopic studies on semiconductor films [6]). Another effect which must be considered is the pressure due to structural rearrangements as evidenced by Parson and coworkers [7–9]. To evaluate the effect of this pressure on the signal of our transducer we carried out measurements with a waffer of fused silica, 1mm thick, behind the $LiNbO_3$ crystal and we placed the leaf disk behind the waffer. The thickness of the waffer is bigger than the thermal diffusion lenght in the fused silica (∼ 0.1 mm at 17 Hz). So, only pressure waves can reach the transducer (the thermal waves were shielded). We choosed the fused silica waffer due the fact that it has pratically the same acoustic impedance of the $LiNbO_3$ ensuring a sound power transmission coefficient close to 1. No signal was observed and we concluded that the pressure contribution to the signal of the transducer is negligible.

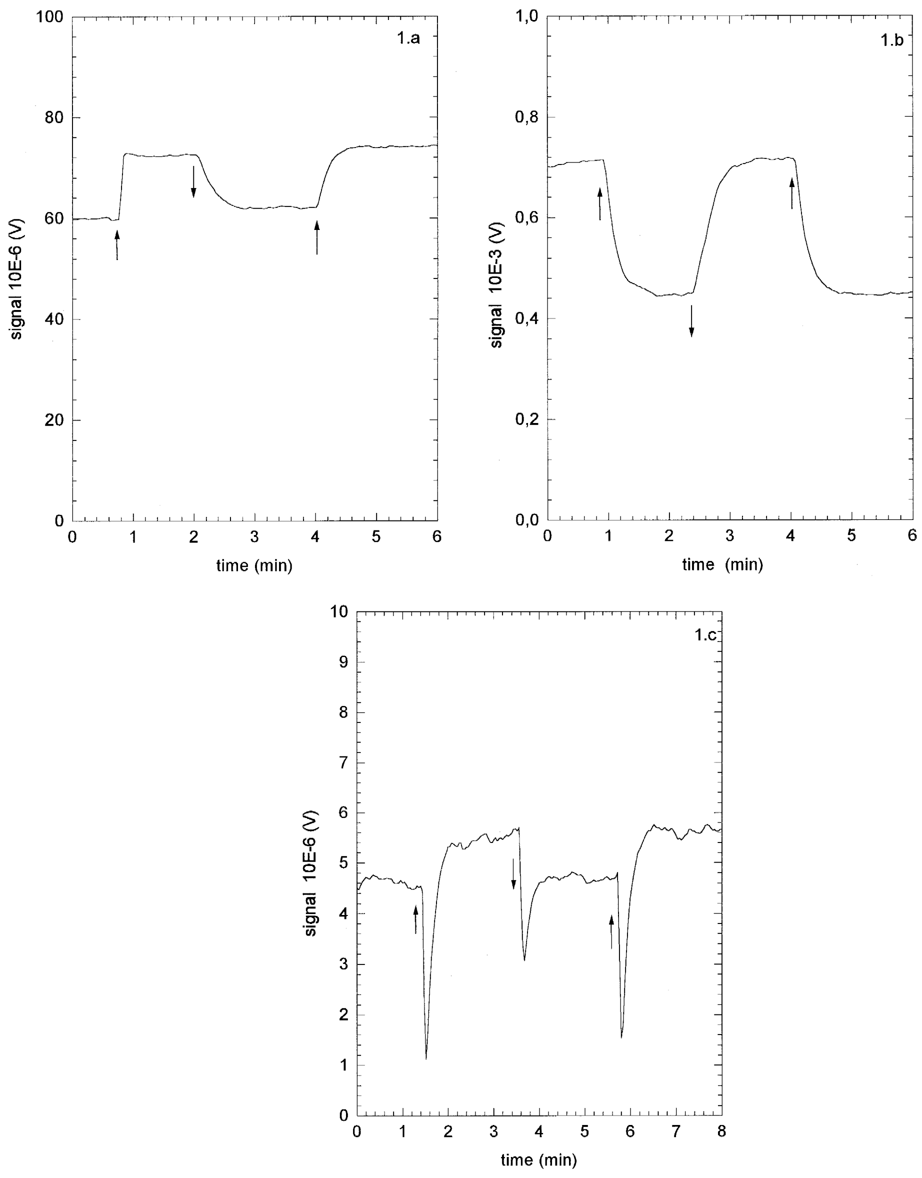

Fig. 1. a: the 'positive' effect obtained with the open-ended photothermal cell at 17 Hz (↑ d.c. light on; ↓ d.c. light off). b: the 'negative' effect obtained with the conventional photoacoustic cell at 17 Hz. c: the 'positive' effect obtained with the conventional photoacoustic cell at 377 Hz.

2. Materials and methods

The spectrometer was composed of a 1000 W Xenon lamp focused onto a 1/4 meter model 77200 Oriel monochromator. At the exit slit of the monochromator the light was modulated by a Stanford Research model 540 mechanical chopper. The measurements were made at 680 nm with a spectral resolution of 9 nm, and the signals were processsed by a dual-phase Stanford Research model 530 lock-in controlled by a microcomputer. The microcomputer takes one measurement at each 2.5 s. The source of the non-modulated light was a d.c. projector coupled with a water filter. To illuminate the sample (a circular leaf disc of *Nicotiana tabacum*) with both a.c. and d.c. lights we used a bifurcated optical fiber bundle (44 W/m^2 modulated light; 580 W/m^2 d.c. light). For the measurements of the light power we used a PZT detector calibrated with an International Light model IL 1700 radiometer. A Bruel & Kjaer model 4166 microphone was used in the photoacoustic cell.

For the open-ended photothermal cell the Eqs. (1–3) of the [1] become:

$$I = I_{th}(1-L) \qquad Q = Q_{th}(1-L) \tag{1}$$

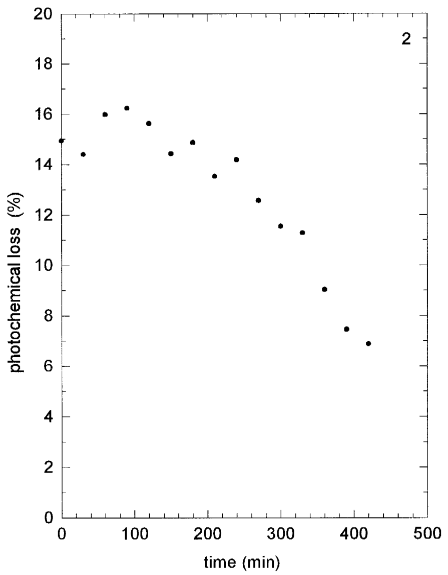

Fig. 2. The behavior of the photochemical loss with time after the leaf was harvested.

Table 1
The behaviour of L with the modulation frequency with the open-ended photothermal cell

Frequency (Hz)	L (%)
17	15.540 ± 0.002
27	14.830 ± 0.002
37	15.248 ± 0.006
47	12.460 ± 0.001
77	15.42 ± 0.02
97	15.19 ± 0.01
377 [a]	16.19 ± 0.02

[a] Photoacoustic cell.

where I and Q are the in-phase and quadrature components, respectively, of the signal for any arbitrary setting of the reference signal. The subscript represents the thermal contribution and L is the photochemical loss. When the non-modulated light is applied the reaction centers are 'closed' saturating photosynthesis and we have:

$$I^* = I_{th} \qquad Q^* = Q_{th} \tag{2}$$

Where the asterisk refers to the presence of non-modulated light. The change in the signal due to the application of non-modulated light is obtained by vectorial subtraction:

$$\Delta I = I^* - I = LI_{th} = LI^* \qquad \Delta Q = Q^* - Q = LQ_{th} = LQ^* \tag{3}$$

the photochemical loss is then given by the ratios:

$$L = \Delta I / I^* = \Delta Q / Q^* = \Delta A / A^* \tag{4}$$

where A is the amplitude of the signal.

3. Results and discussion

Fig. 1a shows the 'positive' effect [1] obtained with the open-ended photothermal cell at 17 Hz and Fig. 1b shows that for the photoacoustic cell one obtains a 'negative' effect [1] at the same frequency. Fig. 1c shows the 'positive' effect obtained with the photoacoustic cell at 377 Hz. As mentioned before the diference between Fig. 1a,b are due to the fact that, differently of the photoacoustic cell, the open-ended photothermal cell is not sensitive to oxygen

Table 2
Successive determinations of the photochemical loss obtained with the phototothermal cell at 17 Hz showing the dispersion of the data

Measurement	Photochemical loss
1	18.285 ± 0.006
2	18.044 ± 0.008
3	16.570 ± 0.004
4	17.015 ± 0.002
5	17.353 ± 0.002
6	17.150 ± 0.003
7	17.649 ± 0.002
8	17.016 ± 0.005
9	15.837 ± 0.003
10	15.450 ± 0.002
Mean	17.0 ± 0.9

evolution. In Fig. 1c one obtains again a ‘positive’ effect because the oxygen evolution was damped out [1]. Fig. 2 shows the behavior of L in function of the time after the leaf was harvested. We see that the L decays slowly with the time what ensures the fidelity of the results shown in this paper. Table 1 shows that L is frequency independent confirming what was supposed in the [1]. Note the high quality of these measurements at low frequencies. In the course of the experiments we noted that the value of the L along the subsequent determinations has a much greater standard deviation than that relative to only one determination. The accuracy is better represented by the variability between repeated acquisitions. These results are shown in Tables 2 and 3 for the open-ended photothermal cell and the photoacoustic cell, respectively. The technique of the determination of L at low frequencies could be used for the determination of the effect of the water stress in plants in a continuous way, since we could make measurements with the leaf still attached to the plant. In view of the good results in the determination of L (see Table 1) we can use with this method with advantage to select heat tolerant species [10]. Presently, is in course in our laboratory a trial to develop a method combining both the photoacoustic and photothermal cell in order to measure absolutely the oxygen evolution in leaves.

Table 3
Successive determinations of the photochemical loss obtained with the photoacoustic cell at 377 Hz showing the dispersion of the data

Measurement	Photochemical loss
1	16.31 ± 0.02
2	13.77 ± 0.01
3	16.42 ± 0.01
4	17.63 ± 0.02
5	16.92 ± 0.01
6	15.57 ± 0.01
7	15.058 ± 0.007
8	15.30 ± 0.02
9	14.670 ± 0.008
10	12.82 ± 0.01
Mean	15.4 ± 1.5

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