A New Device for Simultaneous Measurements of Oxygen Concentration, Absorption and Fluorescence Changes in Photosynthetic Systems

An increasing number of biological studies require the measurement of various spectroscopic and polarographic data with small amounts of material. Especially the simultaneous determination of different responses facilitates the correct interpretation of the results. Such measurements, sometimes already performed in cell suspensions, cause serious difficulties with tissues, e.g. plant leaves.

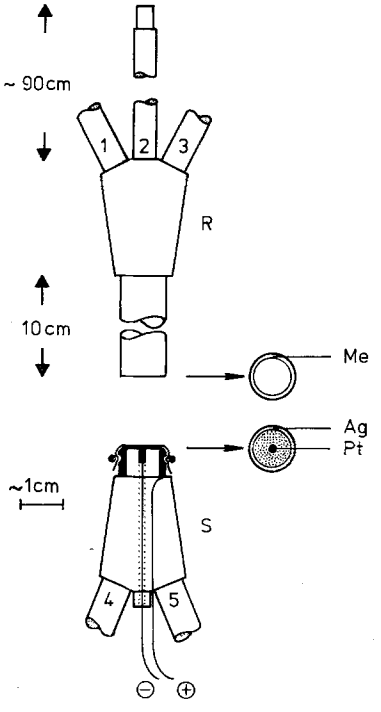

Fig. 1. R: Triple-branched flexible fibre optics connected to a light guiding glass rod. S: Flexible branched fibre optics with central hole for the placement of a platinum wire (Pt). The protecting capsule opposite the branching piece is made of silver (Ag) which serves as reference electrode. The frontal opening is covered by a Teflon membrane fixed by an O ring. As electrolyte between platinum and Ag/AgCl half-saturated KCl is used.

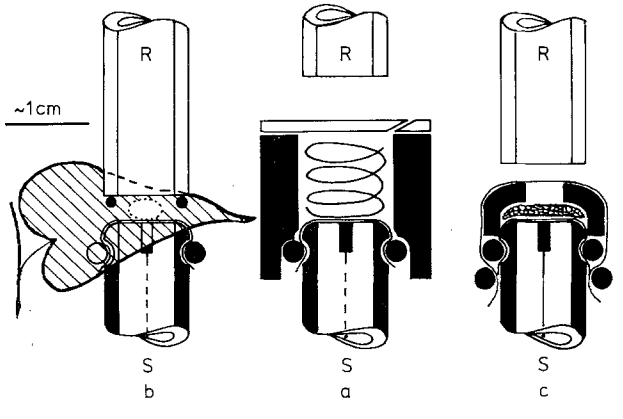

Fig. 2. The application of the wave guide combination R + S for 3 different sample types. a) suspensions; b) leaves; c) tissue samples (details see text).

This communication describes a new set-up allowing the simultaneous determination of absorption, fluorescence and polarographic data with cell suspensions as well as with tissue samples.

Principle. Multiply branched wave guides are used for the optical measurements. For the polaragraphic oxygen determination, a Clark-type electrode has been constructed as part of a fibre optic. The light beam passing to and from the subject is guided to the cell with the electrode combination (Pt/Ag/AgCl).

A sketch of the set-up is given in Figure 1. Two flexible branched wave guides (R and S) opposed to each other are fixed by a microscope tripod. The sample is placed between the 2 ends of the fibre optics. Figure 2 gives 3 examples for the possible arrangement in measurements with a) suspensions (~ 0.5 ml), b) whole leaves (without oxygen determination), or c) tissue samples (with oxygen determination).

To keep the temperature of the sample constant, the set-up can be thermostated by running water. For measurements in suspensions, we replaced the normally used magnetic stirrer by a metal spring which is electromagnetically forced to vibrate. By this means the light beam is never interrupted. Inside the cuvette, another miniature electrode (e.g. for pH determinations) can be fixed. To measure reflection values, the light beam can ofcourse be directed the opposite way.

The ends of the flexible wave guides can be connected to filters, monochromators or photomultipliers. So the apparatus may be quickly combined – without touching the sample – with different optical or electronic instruments. There are practically unlimited possibilities for a combination of mono- or polychromatic excitation with single-, double- or split-beam absorption resp. fluorescence spectroscopy. The application of UV transparent wave guides extends the measurable spectrum into the ultraviolet region. This set-up has been used in our laboratory for several investigations[1-5].

In the near future adaptors will be available which make it possible to connect the wave guides with commercial spectrophotometers, so that their monochromators and multipliers may be directly used.

Zusammenfassung. Klein dimensionierter Küvettensatz für Suspensionen und Gewebe, welcher erlaubt, gleichzeitig verschiedene spektroskopische und elektrochemische Messgrössen zu ermitteln indem Lichtleiter zur Bestimmung optischer Daten eingesetzt werden können.

R. J. STRASSER[6,7]

Department of Botany, Laboratory of Photobiology, University of Liège, B–4000 Sart-Tilman (Belgium), 9 August 1973.

[1] R. J. STRASSER, Verh. Schweiz. Naturforsch. Ges. *71*, 102 (1971).
[2] R. J. STRASSER, VI Int. Congr. on Photobiology, Bochum 1972, Abstracts 252.
[3] R. J. STRASSER and C. SIRONVAL, FEBS Lett. *28*, 56 (1972).
[4] R. J. STRASSER and C. SIRONVAL, Experientia, *29*, 151 (1973).
[5] R. J. STRASSER and C. SIRONVAL, FEBS Lett. *29*, 286 (1973).
[6] The autor thanks Professor H. METZNER and Professor C. SIRONVAL for their support.
[7] Support by EMBO (European Molecular Biology Organization) is gratefully acknowledged. All optical parts of the set-up were produced by the JENA er Glaswerk SCHOTT & GEN. (Mainz/Germany).