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Preparation of Mixtures for the Calibration of Gas Analysers

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ABSTRACT

Differential gas analysers for use in photosynthesis experiments can be calibrated with the same accuracy as that specified for the single standard gas mixture that is required. With the use of only one gas mixing pump, the composition of a mixture can be specified to better than ± 1 per cent, compared with ± 5 per cent usually offered by commercial gas suppliers.

INTRODUCTION

Measurement of the rate of photosynthetic or respiratory carbon dioxide exchange of a leaf usually involves measuring the change in concentration in a gas stream passing over it at a known speed. The minimum speed is set by the need to carry away transpired water in the gas stream, so avoiding condensation in the leaf chamber and allowing the rate of transpiration to be measured. This, in turn, sets the maximum difference, $\Delta\phi_{\max}$, between the volumetric concentrations of carbon dioxide in the gas streams entering and leaving the chamber.

The weight of carbon dioxide fixed in photosynthesis by unit leaf area in ordinary air is usually less than one-hundredth of the weight of water transpired (Bierhuizen and Slatyer, 1965), so that

$$\rho\Delta\phi_{\max} \leq 10^{-2}\Delta\chi,$$

where $\Delta\chi$ is the increase in concentration of water vapour in the gas stream and ρ is the density of carbon dioxide. If the ingoing gas is dried to a dew-point of 0°C and the outgoing gas is saturated at 20°C , $\Delta\chi = 13 \times 10^{-6} \text{ g cm}^{-3}$, giving $\Delta\phi_{\max} \leq 65 \times 10^{-6} \text{ cm}^\circ (\text{v/v})$. Saturation is usually avoided and $\Delta\phi \leq 50 \times 10^{-6} \text{ cm}^\circ$ in most experiments. Differential infra-red or conductimetric gas analysers are suitable for measuring concentration differences of this size, but accurate calibration has so far been difficult.

A differential infra-red carbon dioxide analyser with a full-scale deflection of $50 \times 10^{-6} \text{ cm}^\circ$ can be calibrated at any particular concentration by the divided-tube method of Parkinson and Legg (1971) with the same accuracy as that specified for the single standard gas mixture that is required. As the analyser sensitivity varies with gas concentration, a range of standard gas mixtures is required if the relation between photosynthesis rate and carbon dioxide concentration is to be studied.

Two conductimetric carbon dioxide analysers can be connected to make a differential instrument (Lake and Begg, 1971) and, if the analyser sensitivities are matched, the calibration is linear and independent of the absolute concentration of the gases in a range that depends on the instrument specification, usually $0\text{--}600 \times 10^{-6} \text{ cm}^{\circ}$. Thus the calibration can be made by comparing pure nitrogen and a single standard gas mixture; once again the accuracy is the same as that specified for the standard gas mixture.

Suppliers of gas cylinders containing small ($< 10^{-2} \text{ cm}^{\circ}$) concentrations of carbon dioxide in nitrogen usually specify the concentration to ± 5 per cent of the stated value (Bate, D'Aoust, and Canvin, 1969; Parkinson and Legg, 1971) and such poor accuracy is unacceptable for most experiments. An alternative is to mix carbon dioxide and nitrogen using either volumetric flow meters, when the inaccuracy is about ± 4 per cent (Parkinson, 1968), or gas mixing pumps, for which the makers claim excellent accuracy (± 0.24 per cent or better). However, the price of one gas mixing pump is about the same as that of a differential conductimetric analyser, or one third of the price of an infra-red analyser. To produce gas mixtures in the range of interest for photosynthesis experiments (say 0 to $10^{-3} \text{ cm}^{\circ}$), at least two pumps are usually used in series; Ludlow and Jarvis (1971) used three, Bate *et al.* (1969) used four in a series/parallel arrangement to calibrate a differential analyser.

The method described requires only one gas mixing pump and gives an overall accuracy of ± 0.9 per cent.

METHOD

Cylinders of pure nitrogen and carbon dioxide, one cylinder (*C*) containing a carbon dioxide/nitrogen gas mixture, one mixing pump and one absolute conductimetric gas analyser are used. First, gas from the cylinder *C* is compared by means of the analyser with a mixture of pure carbon dioxide and nitrogen from the pump, so that the composition of the cylinder gas can be accurately calculated. Then the pump is used to dilute the cylinder gas further to provide concentrations suitable for the calibration of differential gas analysers.

The gas mixing pump (H. Wösthoff, Bochum, Germany; type SA 27/3F) was capable of delivering mixtures containing fractions of carbon dioxide in nitrogen ranging from 0.01 to 0.10 cm° , at 10–20 mbar above atmospheric pressure so that the mixture could be delivered to a conductimetric analyser.

The accuracy of the pump was tested with the fraction set at 0.10. A Hyde water-sealed rotating drum gas meter (A. Wright & Co. Ltd.) with a response of four revolutions per litre and an accuracy of ± 0.25 per cent was used to measure the rates of flow of the two gases into the pump and the mean pump fraction was found to be correct within the accuracy of the gas meter.

The cylinder of commercial gas mixture contained a nominal concentration of $0.0105 \text{ cm}^{\circ}$ of carbon dioxide in nitrogen; as the supplier's claimed accuracy was ± 5 per cent, this specification ensured that the real concentration, ϕ_{cyl} , exceeded $0.0100 \text{ cm}^{\circ}$. An absolute conductimetric analyser, adjusted electrically to the appropriate sensitivity, was calibrated against fractions of this mixture, diluted with nitrogen, in the range 0.90 to 1.00. The output of the analyser when supplied with a mixture of $0.0100 \text{ cm}^{\circ}$ of carbon dioxide in nitrogen from the mixing pump intersected the calibration curve at $0.922 \phi_{\text{cyl}}$, so that $\phi_{\text{cyl}} = 0.01085 \text{ cm}^{\circ}$.

Gas from the cylinder could then be diluted with nitrogen by means of the pump to give mixtures ranging from 108.5×10^{-6} to $1085 \times 10^{-6} \text{ cm}^{\circ}$, suitable as standards for calibrating differential analysers by the methods mentioned in the Introduction.

The cylinder also provided a useful reference against which to test other mixtures. For

example, the sensitivity of the absolute conductimetric analyser was increased and it was calibrated in suitable ranges such as $0.02 \phi_{\text{cyl}}$ to $0.04 \phi_{\text{cyl}}$ by means of the mixing pump, to enable it to be used to measure the concentrations in several cylinders bought from suppliers (Table 1). Suppliers' analyses seem notably inaccurate at large concentrations.

TABLE 1. *Comparison of analyses of carbon dioxide/nitrogen mixtures bought from three suppliers*

Supplier	Concentration $10^{-6} \text{ cm}^{\circ}$		
	Ordered	Supplier's analysis	Measured value
1	250	..	260
2	200	200	201
2	300	300	300
2	400	400	393
3	300	300	304
3	380	380	374
3	650	617	703
3	900	980	995
3	1600	1620	1700

ACCURACY

The output of the conductimetric analyser was displayed on a potentiometric recorder and could be reproduced to $\pm 5 \mu\text{V}$, corresponding to ± 0.4 per cent of the absolute gas concentration. Estimation of the concentration in a mixture prepared for calibration purposes by diluting the cylinder gas includes the accuracy of the gas mixing pump twice, so that the over-all accuracy is $\pm(0.4 + 2 \times 0.24 \text{ per cent}) = \pm 0.9$ per cent.

When an absolute analyser, calibrated with one of these mixtures, is used to measure the concentration of other gas mixtures, the analyser accuracy must be added again, giving a value of ± 1.3 per cent.

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