

# A Numerical Approach to Measurement of CO<sub>2</sub> Exchange Transients by Infrared Gas Analysis

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## ABSTRACT

Open flow-through systems coupled to infrared gas analyzers have been frequently employed in the study of CO<sub>2</sub> exchange transients such as the postillumination burst observed in leaves of C<sub>3</sub> plants. A major limitation associated with use of such systems is their non-steady state response to rapid changes in leaf CO<sub>2</sub> exchange rate. A previous publication outlined a numerical approach to model the analyzer response as a function of CO<sub>2</sub> exchange rate and thus permit estimation of the postillumination burst (Peterson 1983 Plant Physiol 73: 978-982). The model is critically analyzed within the framework of the physics of solute dispersion as previously described for linear flow systems. Thus, the numerical simulation is validated on the basis of physical principle. Additional improvements to the previous model are described which enhance the accuracy and efficiency of use of this technique for estimation of photorespiration.

$$\sigma_x^2 = \frac{a^2 u^2}{24D} t \quad (1)$$

where  $\sigma_x^2$  is the variance of the distribution along the direction of flow,  $a$  is the radius of the tube,  $u$  is the mean velocity of flow,  $D$  is the cross-stream mixing coefficient for solute, and  $t$  is the time. The maximum values for  $\sigma_x^2$  is given using the molecular diffusivity of air for  $D$  [ $D \approx 0.15 \text{ cm}^2 \text{ s}^{-1}$ ] in equation 1. For this system,  $a = 0.95 \text{ cm}$ ,  $u = 5.7 \text{ cm s}^{-1}$ , and  $L$ , the length of the tube, is about 34 cm. Thus, the increase in the variance of the distribution as gas passes through the tube,  $\Delta\sigma_x^2$ , is constrained by:  $\Delta\sigma_x^2 \leq 49 \text{ cm}^2$ . This dispersion due to the cross-sectional variation in velocity can be modelled by a finite difference scheme utilizing the phenomenon of numerical dispersion. The tube may be simulated by a series of compartments of equal volume through which the fluid passes with uniform velocity. Figure 1 illustrates how such a model may simulate dispersion of solute during flow. The final solute concentration ( $C$ ) in any compartment ( $i$ ) over time interval  $\Delta t$  may be expressed in terms of the flow rate ( $F$  in  $\text{l s}^{-1}$ ) and compartment volume ( $V$  in liters).

$$C_i(t_2) = C_i(t_1) + \frac{F\Delta t}{V} [C_{(i-1)}(t_1) - C_i(t_1)] \quad (2)$$

The PIB<sup>1</sup> of CO<sub>2</sub> in C<sub>3</sub> leaves has been recognized as an index of the rate of metabolism of glycolate. In two previous reports, a non-steady state approach was described for quantitative estimation of the PIB using an IRGA equipped with a flow-through cell (1, 2). A numerical formula was employed to simulate the dispersion of CO<sub>2</sub> as it passed through the tubular cell of the IRGA. This paper presents theoretical evidence to justify use of the previous basic model. Refinements to the basic model improve accuracy and increase the efficiency of determining optimal values for critical model parameters.

## MATERIALS AND METHODS

Details of the apparatus and other experimental conditions have been described in Peterson (1). Tube dimensions apply to the Beckman 865 IRGA sample cell (length, 34.35 cm; diameter, 1.9 cm).

## RESULTS AND DISCUSSION

**The IRGA Response Model.** As a fluid passes through a cylindrical tube, the radial velocity profile is not uniform. Flow is most rapid at the center and zero at the wall. The streamwise (*i.e.* along the  $x$  axis) dispersion of a solute in a bounded shear flow has been extensively studied (4-6). If the time to mix across the flow is less than or comparable to the time to pass through the tube (for this system both time scales are about 6 s), then:

The degree of dispersion is strongly dependent upon the value of  $\Delta t$ . To study the effect of numerical dispersion, we introduce the dimensionless Courante number (3),  $p = F\Delta t/V = u\Delta t/\Delta x$ , which is the fraction of material which moves from one compartment to the next in one time step (Fig. 1). Equation 2 then becomes:

$$C_i(t_2) = (1 - p)C_i(t_1) + pC_{i-1}(t_1) \quad (3)$$

The value of  $p$  also represents the probability of advancing a distance  $\Delta x$  in time  $\Delta t$  whereas  $(1 - p)$  is the probability of stasis. If we assume that at time zero all of the material is in the zeroth compartment at concentration  $C_0$ , then the concentration in the  $m$ th box after  $n$  time steps, such that  $t = n\Delta t$ , is:

$$C_{i+m}(t_n) = C_0 \frac{n!}{(n-m)!m!} p^m (1-p)^{n-m}, \quad (4)$$

which is simply the binomial distribution (Fig. 2). The mean number of boxes moved is  $np$  and the variance about this mean is  $\sigma^2 = np(1-p)$  at any time  $t = n\Delta t$ . In terms of distance, the mean is  $\bar{x} = np\Delta x = F\Delta x t/V = ut$  and the variance  $\sigma_x^2$  is given by:

$$\sigma_x^2 = \Delta x^2 np(1-p) = \Delta x ut \left(1 - \frac{u\Delta t}{\Delta x}\right) \quad (5)$$

For a system of eight compartments (1),  $\Delta x = L/8$  and the increase in the variance of the distribution on passing through the eight compartments is:

<sup>1</sup> Abbreviations: PIB, postillumination burst; IRGA, infrared gas analyzer.

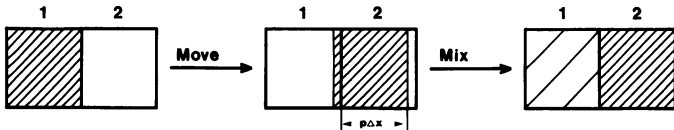


FIG. 1. Example using a two-compartment model showing how dispersion of material in a fluid stream may be simulated numerically. Solute (cross-hatched) moves uniformly a distance given by the product of the compartment length ( $\Delta x$ ) and  $p$ , the Courante number. See text for further details.

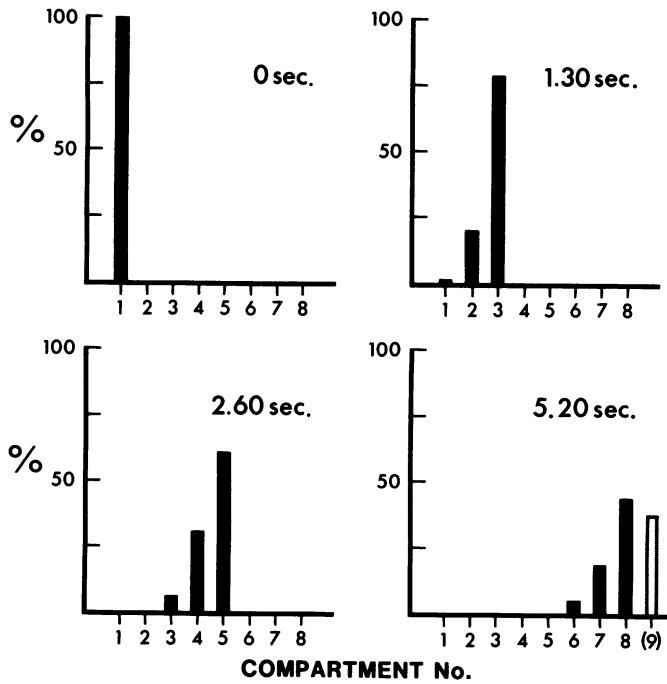


FIG. 2. Simulated distribution of CO<sub>2</sub> versus length during flow through the sample cell of a Beckman 865 IRGA (total volume = 97.76 ml). The cell is simulated by eight serially connected compartments (each 12.22 ml), the first of which contains CO<sub>2</sub> at time zero. The incoming gas is CO<sub>2</sub>-free, the flow rate is 1.0 l min<sup>-1</sup>, and  $\Delta t = 0.65$  s. Ordinates are expressed as per cent of the CO<sub>2</sub> concentration initially present in the first compartment. Note that no CO<sub>2</sub> is lost until step 8 (5.20 s when CO<sub>2</sub> first leaves the cell). This is depicted in the lower right panel where the CO<sub>2</sub> content in an imaginary ninth compartment is shown. The binomial nature of the distribution (Eq. 4) is apparent.

$$\Delta \sigma_x^2 = 144 \text{ cm}^2(1 - \Delta t/0.75 \text{ s}) \quad (6)$$

A comparison of equations 6 and 1 yields  $\Delta t \geq 0.495$  s. In model experiments in which a discrete quantity of CO<sub>2</sub> was injected into the flowing gas stream, satisfactory fits between predicted and observed IRGA responses were obtained for  $\Delta t > 0.495$  s. A value of 0.65 s for  $\Delta t$  has been routinely employed in experiments. The somewhat enhanced cross-stream mixing associated with  $\Delta t = 0.65$  s versus 0.495 s is reasonable since inlet and outlet ports are placed laterally on the cylinder and the air flow had to twice negotiate 90° turns. These bends induce turbulent mixing and probably increase the effective value of  $D$  (Eq. 1).

**Application of the Model to Estimation of PIB.** As stated previously (1), changes in CO<sub>2</sub> concentration ( $C_2$  in  $\mu\text{l l}^{-1}$ ) in the well-mixed leaf chamber located upstream from the IRGA cell may be written as a function of the incoming CO<sub>2</sub> concentration ( $C_1$ ), flow rate ( $F$ ), chamber volume ( $V_2$ ), and the rate of net CO<sub>2</sub> exchange  $R(t)$  (in  $\mu\text{l s}^{-1}$ ).

$$dC_2 = \frac{F(C_1 - C_2) dt - R(t) dt}{V_2} \quad (7)$$

In general, net CO<sub>2</sub> exchange ( $R(t)$ ) is the sum of gross CO<sub>2</sub> fixation ( $R_G$ ) and internal CO<sub>2</sub> evolution ( $R_E$ ) or  $R(t) = R_G(t) + R_E(t)$  [CO<sub>2</sub> release is (-), CO<sub>2</sub> uptake is (+)]. The steady state rate of net uptake of CO<sub>2</sub> in the light ( $R'$ ) continues unabated for a few seconds following darkening of a leaf, then CO<sub>2</sub> uptake rapidly declines. This is followed by a net surge of CO<sub>2</sub> which declines until a relatively stable rate of CO<sub>2</sub> release ( $K$ ) is reached by 30 to 60 s after darkening (1, 2). The steady state rates of CO<sub>2</sub> evolution and gross photosynthesis in the light,  $R_E'$  and  $R_G'$ , may be expressed in terms of  $R'$ ,  $K$ , and an arbitrary constant  $A$ . Thus,  $R_E' = K - AR'$  and  $R_G' = R' + AR' - K$ . Values for  $R'$  and  $K$  are obtained directly from the data using the steady state relationship  $R = F(C_1 - C_2)$ . The leaf chamber is connected to the IRGA by narrow inlet tubing through which gas passes with little mixing adding a  $\sim 1.7$ -s delay to the IRGA response. This delay plus the real delay in the decline of net CO<sub>2</sub> uptake after darkening constitutes the overall delay time  $S_1$  (s). Subsequently, both  $R_E$  and  $R_G$  are assumed to decline exponentially such that for any  $t > S_1$

$$R_G(t) = (R' + AR' - K)e^{-B_1(t-S_1)} \quad (8)$$

$$R_E(t) = K - (AR')e^{-B_2(t-S_1)} \quad (9)$$

Substitution of  $R_G(t) + R_E(t)$  for  $R(t)$  in equation 7 followed by integration over the limits  $t_1$  to  $t_2$  yields a general expression for the chamber CO<sub>2</sub> concentration at any  $t \geq S_1$  following darkening.

The change in CO<sub>2</sub> concentration ( $C_3$ ) in the first compartment (of volume  $V_3$ ) of the IRGA cell is described by

$$\int_{t_1}^{t_2} dC_3 = \int_{t_1}^{t_2} \frac{FC_2(t)}{V_3} dt - \frac{FC_3 \Delta t}{V_3} \quad (t \geq S_1) \quad (10)$$

Predicted CO<sub>2</sub> concentration changes over any  $\Delta t$  for the remaining compartments are calculated using equation 2. The predicted IRGA response at the end of any  $\Delta t$  is simply the mean of the CO<sub>2</sub> concentrations in the eight compartments of the IRGA cell.

Thus, four adjustable parameters ( $S_1$ ,  $A$ ,  $B_1$ ,  $B_2$ ) are employed in determining the time course of  $R(t)$  such that predicted and observed IRGA responses are in maximal agreement (2). The maximum rate of CO<sub>2</sub> evolution, which usually occurs at 6 to 10 s after darkening, is taken as an estimate of  $R_E'$ .

We wish to contrast the current analysis with the numerical simulation described previously (1, 2) in which the  $\Delta t$  used was quite short (0.025 s). First, for  $\Delta t > 0.37$  s in this system (*i.e.*  $\Delta t$  greater than  $\frac{1}{2}$  times the flush time of one IRGA cell compartment) the distribution of CO<sub>2</sub> in the IRGA cell during flow is skewed backward relative to the direction of flow instead of forward ( $\Delta t < 0.37$  s). This is consistent with the expected effect of the nonuniform cross-sectional velocity profile in the cell on the distribution of CO<sub>2</sub> (see above). Second, a larger  $\Delta t$  dramatically increases the efficiency of obtaining optimal values for the four adjustable parameters using a reiterative computerized search procedure (2). Third, utilizing the integrals of equations 7 and 10 in this model, instead of finite difference equations as used previously (1, 2), results in total conservation of CO<sub>2</sub> and thus an improvement in accuracy. Fourth, an improved continuous formulation for leaf CO<sub>2</sub> exchange rate  $R(t)$  (Eqs. 8 and 9) yields a more reliable estimate of the maximum rate of postillumination CO<sub>2</sub> evolution. The previous function for  $R(t)$  exhibited a discontinuity occurring between the cessation of net CO<sub>2</sub> uptake and the onset of net CO<sub>2</sub> evolution.

Since the PIB is highly O<sub>2</sub>-dependent (2),  $R_E'$  is considered to arise primarily from the metabolism of glycolate to CO<sub>2</sub> (photorespiration). Table I shows the typical response of the photorespiration: net photosynthesis ratio in tobacco leaf discs to increasing O<sub>2</sub> concentrations. Analyses of variance of predicted versus observed IRGA responses indicated that an average of only 0.018% of the total sum of squares in each determination was

Table I. Effect of O<sub>2</sub> on the Ratio of Photorespiration to Net CO<sub>2</sub> Uptake in Tobacco Leaf Discs

Samples containing ten 1.6-cm discs (inverted, 0.24 g fresh wt) were preilluminated at 1000 to 1100  $\mu\text{E m}^{-2} \text{s}^{-1}$  until steady state photosynthesis was obtained. The temperature was 32°C. The average flow rate was 0.01654 l s<sup>-1</sup>, the leaf chamber gas phase volume was 0.099 L, and the initial CO<sub>2</sub> concentration was 336 to 354  $\mu\text{l l}^{-1}$ . Other details of the apparatus are in Peterson (1, 2). Note that the experiment was performed in duplicate on the same sample at each O<sub>2</sub> level. Four separate yet similar samples were employed, one for each O<sub>2</sub> concentration. Thus variation is attributed to differences among these samples and is evident especially regarding the effect of O<sub>2</sub> on net photosynthesis. Clearly, however, photorespiration is enhanced relative to net photosynthesis as the ambient O<sub>2</sub> concentration is increased as measured using the model described in the text.

O <sub>2</sub>	S <sub>1</sub>	A	B <sub>1</sub>	B <sub>2</sub>	Net Photosynthesis	Photorespiration	Photorespiration Net Photosynthesis
%	s		s <sup>-1</sup>		$\mu\text{mol CO}_2 \text{ g fresh wt}^{-1} \text{ h}^{-1}$		
16	4.8	-0.05	1.5781	0.0219	177.8	26.5	0.149
21	5.0	0.25	1.3828	0.2000	163.6	50.8	0.310
30	5.1	0.375	1.2969	0.0735	66.9	50.8	0.760
40	5.0	0.500	1.5937	0.0594	69.8	61.6	0.882

unaccounted for using the model described herein. Also, six independent experiments performed under atmospheric levels of O<sub>2</sub> and CO<sub>2</sub> (temp. = 32°C) produced a mean ratio of photorespiration:net photosynthesis of  $0.283 \pm 0.028$  (SE). Duplicate determinations within an experiment frequently agree closely (*i.e.*  $\pm 5\%$ ). This approach produces valid and reasonable estimates of photorespiration:net photosynthesis and should aid in future studies of the regulation of photorespiration in C<sub>3</sub> plants.

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