Measurement of CO$_2$ Dissolved in Aqueous Solutions Using a Modified Infrared Gas Analyzer System[1]

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ABSTRACT

Total dissolved inorganic carbon (ΣCO_2) and aqueous carbon dioxide (H_2CO_3*) in nutrient solutions may be measured by the injection of small gas or liquid samples (1 microliter to 8 milliliters) into a gas stripping column connected in-line with an infrared gas analyzer. The measurement of ΣCO_2 in solution requires sample acidification, while H_2CO_3* and gaseous CO_2 are measured without the addition of lactic acid. The standard curve for ΣCO_2 was linear up to 300 nanomoles CO_2. Maximum sensitivity was approximately 300 picomoles. Measurements of H_2CO_3* were independent of pH. Consequently, ΣCO_2 and H_2CO_3* could be used to calculate the pH, HCO_3^-, and CO_3^{2-} values of nutrient solutions. Injection and complete analyses required from 0.8 to 2 minutes.

The measurement of CO$_2$ dissolved in aqueous solutions is complicated by the reaction of CO$_2$ with water to form carbonic acid (H$_2$CO$_3$) and the associated protolysis anions, bicarbonate (HCO$_3^-$) and carbonate (CO$_3^{2-}$). This reaction results in the interdependence of the aqueous solute components H$_2$CO$_3$* (representing the moieties of CO$_2$ [aq] and H$_2$CO$_3$ where only a small fraction of CO$_2$ [aq] occurs as H$_2$CO$_3$), HCO$_3^-$, CO$_3^{2-}$, H$^+$, and OH$^-$ at equilibrium. The concentration of H$_2$CO$_3$* is related to the pCO$_2$ of the gaseous phase in equilibrium with the liquid phase by [H$_2$CO$_3$*] = α pCO$_2$, where α is the solubility coefficient for CO$_2$ in water at a specific temperature. Consequently, measurements of dissolved CO$_2$ are a function of the pH, pCO$_2$ of the equilibrium gas phase, carbonate alkalinity ([HCO$_3^-$] + 2[CO$_3^{2-}$]), temperature and the ionic strength of the solution (7, 8). Any method used to measure CO$_2$ dissolved in solution must take into consideration the implications of the reaction of CO$_2$ with water.

Numerous methods have been developed to measure ΣCO_2^2 (1, 7, 9). Indirect methods are based on the measurement of any two of the following parameters: pCO$_2$, pH, and carbonate alkalinity. Direct measurements of ΣCO_2 essentially require the acid conversion of bicarbonate and carbonate ions to CO$_2$, the separation of gas and liquid phases, and the quantitative measurement of the released CO$_2$ gas. These methods require a considerable amount of time or relatively complex modifications of gas chromatographic equipment. This report describes an inexpensive modification of the technique reported by Clegg (2) which improves on other IRGA methods used to measure dissolved CO$_2$ (3, 5, 7). A

[2] Abbreviations: ΣCO_2, total inorganic carbon; IRGA, infrared gas analyzer; GSC, gas stripping column.

GSC similar to that described by Swinnerton (9) was used to remove CO$_2$ from solutions.

MATERIALS AND METHODS

A rapid IRGA technique for measuring CO$_2$ (2) was modified by incorporating an integrating microprocessor and a GSC (Fig. 1). The integrating microprocessor was adjusted to measure the area under the peak and convert the area to μg or nmol of CO$_2$. Flow rate of the N$_2$ carrier gas was maintained at 0.6 l/min.

Gas Stripping Column. The GSC used to remove dissolved CO$_2$ from sample solutions (Fig. 1) is similar to other systems reported for the GLC analyses of CO$_2$ (9). The GSC consisted of a glass chromatographic tube 1 cm in diameter with a coarse glass frit 5 cm from the tip of the tube. A standard removable septum was attached to the chromatographic column at a 45° angle 1 cm above the glass frit. Glass and rubber vacuum tubes connected the GSC to the IRGA sample line. Liquid or gas samples ranging from 1 to 8,000 μl were injected into the GSC using microsyringes (Hamilton type) or plastic surgical-type syringes. Side injection near the glass frit resulted in an even exposure of the sample liquid to the stripping gas. The sample was removed through the bottom of the GSC by the use of a vacuum trap (Fig. 1).

ΣCO_2 and H_2CO_3* Measurement. Prior to a series of measurements, the IRGA system was calibrated by the injection of standards. Measurement of ΣCO_2 involved an initial injection of 0.5 ml of 0.1 N lactic acid into the GSC. The amount and concentration of the acid could be varied according to the alkalinity and volume of the sample. Generally, multiple measurements could be made using one injection of acid. Sample injection was made after CO$_2$ dissolved in the lactic acid was removed by the CO$_2$-free N$_2$ stripping gas flowing at a rate of 0.4 l/min. Sample volume could be varied based on the approximate ΣCO_2 in the sample. The surfactant qualities of lactic acid also improved the efficiency of separating gases from the solution by reducing the size of gas bubbles in the GSC. Total time between injections required from 1 to 2 min and was reduced if reacidification was not required. Standards were prepared from μl injections of a 1 mM Na$_2$CO$_3$ solution (1).

The procedure for H$_2$CO$_3$* measurement involved initial rinsing of the GSC with distilled H$_2$O to remove residual acidity. Sample manipulation was similar to that described for the ΣCO_2 measurement except there was no acidification. Standards were prepared by equilibrating aqueous solutions with gas from commercially prepared standards of 225, 339, and 635 μl/l pCO$_2$ (Matheson) at known temperatures. Standard curves were constructed for H$_2$CO$_3$* using the relationship: H$_2$CO$_3$* = α pCO$_2$ where α is the solubility coefficient of CO$_2$ in distilled H$_2$O. Solutions of known pCO$_2$, pH, and temperature were prepared and analyzed. Calculations were based on the appropriate equations used to calculate the various ΣCO_2 components. Comparisons were made with measurements made by the modified IRGA system.
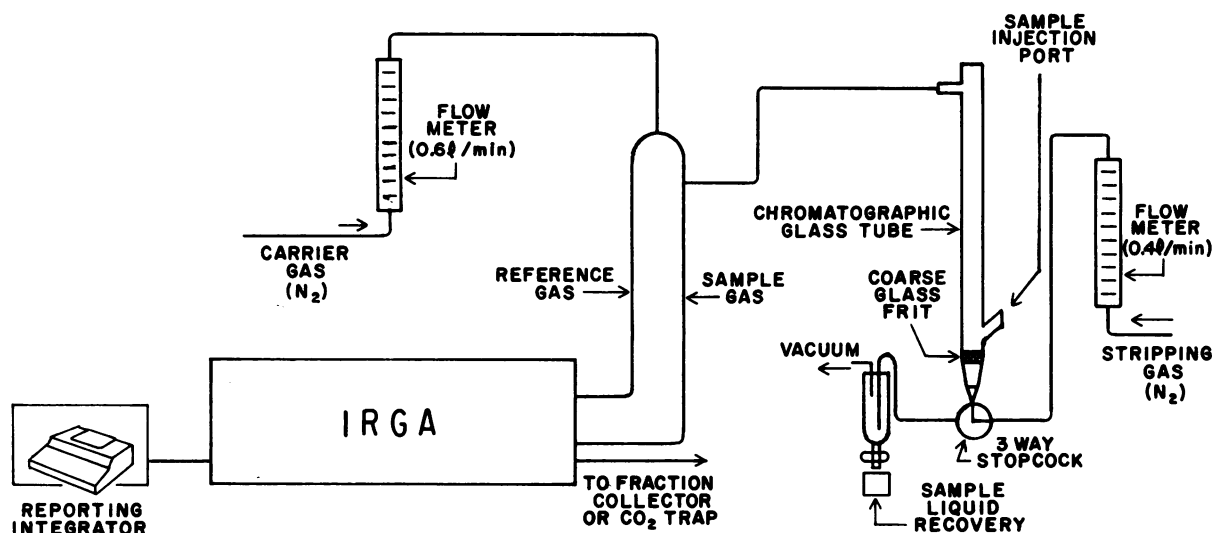
FIG. 1. Diagramatic representation of the system used to measure CO₂ in gas and liquid samples.

RESULTS AND DISCUSSION

Injection of μl quantities of Na_2CO_3 for the ΣCO_2 standard curve resulted in a linear response up to 300 nmol CO_2 (Fig. 2). Quantities greater than 300 nmol CO_2 overloaded the IRGA detection system. The minimum limit of detection achieved was 300 pmol CO_2. This sensitivity approximates the detection limits achieved by the GLC-methanation technique (1) and is at least three orders of magnitude more sensitive than the detection limits of thermal conductivity or previous IR analyzer methods for measuring total CO_2 dissolved in solution. The coefficient of variation at 2 nmol of CO_2 was 7.1% ($n = 10$). Coefficients of variation for larger quantities of CO_2 were generally in the range of 0.5 to 4.0% ($n = 6$). The coefficients of variation for the CO_2 determinations reported in thi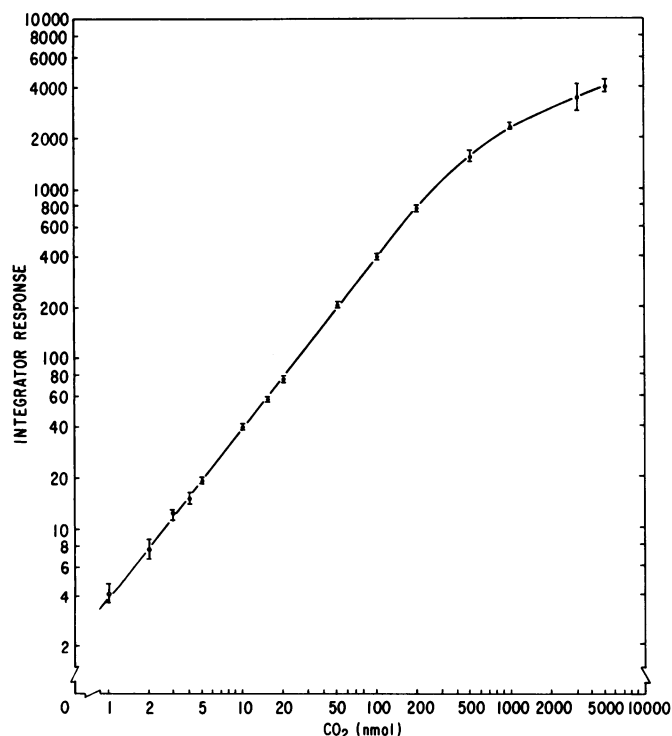s paper are comparable to those observed by Jensen (4) for CO_2 determinations by the tonometer and barium hydroxide methods.

The H_2CO_3* standard curve was similar to the ΣCO_2 standard curve. Chemical equilibrium analysis of the relationship between pCO_2 and H_2CO_3* indicates that for an open system, the $[H_2CO_3*]$ should be independent of ΣCO_2 and pH. This was verified by the experiment summarized in Table I in which the pH values of the test solutions were varied. A comparison of IRGA responses from acidified samples (ΣCO_2) and nonacidified samples (H_2CO_3*)

Table I. *Effect of Varying the pH on ΣCO_2 and $[H_2CO_3*]$ under Constant pCO_2, Ionic Strength, and Temperature*

$pCO_2 = 225$ μl/l, I = 30 mM, T = 23°C. Values represent the means of six determinations $\pm$ SD.

pH	[H₂CO₃*]	ΣCO₂
	μM	
5.09	7.7 ± 0.3	7.9 ± 0.2
5.91	8.3 ± 0.3	11.7 ± 0.5
6.95	7.6 ± 0.9	43.0 ± 0.7
7.72 .	9.0 ± 2.2	229.9 ± 3.2

FIG. 2. CO₂ standard curve based on μl injections of 1 mM Na₂CO₃. Vertical lines represent the 95% confidence limits for the mean value of six determinations.
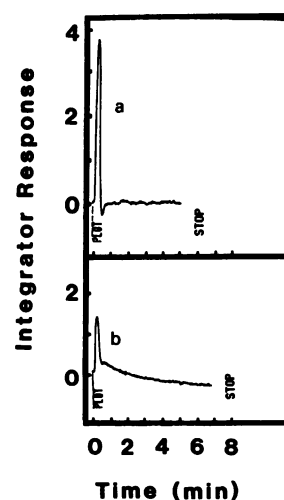

FIG. 3. Comparison of acidified and nonacidified sample peaks. All conditions are similar except for the acidification of the sample. a, Sample injection into 0.1 N lactic acid in the GSC. b, Sample injection into acid-free GSC.

Table II. *Comparison of Four Methods for Determining the* ΣCO_2 *and Carbonate Components of Hoagland Solution of Known Ionic Strength and Temperature (I = 30 mM, T = 20 ± 0.1°C) for two pH Levels*

Values for measured parameters represent the means of six determinations.

Measured Parameters	pH	$H_2CO_3^*$	HCO_3^-	CO_3^{2-}	$\Sigma CO \pm$ SD
				μM	
pH, pCO_2[a]	6.08	24.1	13.9	1×10^{-4}	38.1 ± 1.8
$H_2CO_3^*$, ΣCO_2[b]	6.16	23.7	16.7	2×10^{-4}	40.5 ± 0.5
pH, ΣCO_2	6.08	25.6	14.9	1×10^{-4}	40.5 ± 0.5
pH, $H_2CO_3^*$	6.08	23.7	13.7	1×10^{-4}	37.5 ± 2.9
pH, pCO_2	6.82	24.1	79.3	5×10^{-2}	103.6 ± 4.9
$H_2CO_3^*$, ΣCO_2	6.91	22.4	87.0	5×10^{-2}	109.5 ± 1.3
pH, ΣCO_2	6.82	26.3	83.2	5×10^{-2}	109.5 ± 1.3
pH, $H_2CO_3^*$	6.82	22.4	72.7	5×10^{-2}	95.2 ± 11.2

[a] pCO_2-Matheson standard gas mixture (635 μl/l).
[b] $H_2CO_3^*$ and ΣCO_2 measured by the modified IRGA-GSC system.

indicated a symmetrical peak for the acidified sample (Fig. 3). The asymmetric tailing of the nonacidified sample indicated that a slower conversion of HCO_3^- to CO_2 occurred at near neutral pH values (5, 6). Nutrient solution concentrations had essentially no affect on the measurement of ΣCO_2 and $H_2CO_3^*$ concentrations in Hoagland solutions for concentrations of 15, 30, and 60 mM (one-quarter, one-half, and full-strength modified Hoagland solutions, respectively). The ΣCO_2 measured by our modified IRGA system correlated well with ΣCO_2 values calculated from aqueous solutions of known pH, temperature, and pCO_2 at constant ionic strength (Table II). In addition, relative standard deviations (s) calculated according to Wäser (10) were lower when measuring the ΣCO_2 by our modified IRGA system (s = 0.45) than were the calculated values for ΣCO_2 based on measured values for pH, temperature, and reported pCO_2 values (s = 1.14).

The CO_2-carbonate system is described at equilibrium by a series of equations based on concentrations of the appropriate solute components balanced for electroneutrality and on equilibrium constants for specific temperatures and ionic strengths (7, 8). The appropriate equations may be solved for equilibrium concentrations of all components by utilizing two or more of the four measurable concentration parameters (pH, carbonate alkalinity, pCO_2, and ΣCO_2) with appropriate equilibrium constants. Table II compares four systems of measurements used to calculate the concentration of all CO_2-carbonate solute components from Hoagland nutrient solutions having two pH levels. These results indicate the interchangeability of pH, $H_2CO_3^*$, and ΣCO_2 for use in determining carbonate solute components. An analysis of relative errors using the present techniques for CO_2 and pH measurement indicate that the smallest s value for all component calculation-would be from the pH and ΣCO_2 combination. The $H_2CO_3^*$ and ΣCO_2 combination could be used to calculate pH values in

situations where this might be difficult as in samples of low volume.

Jensen (4) outlines the advantages of using closed systems for measuring root respiration. These systems reduce the requirements for constant conditions of pH and temperature needed for gas equilibrium in open systems. The described IRGA method allows the rapid measurement of changes in CO_2 dissolved in closed systems in which root systems are surrounded by circulating nutrient solution. The method is nondestructive and additional gases removed by the stripping system could be analyzed by connecting other analytical systems to the IRGA. The system also permits the trapping of labeled $^{14}CO_2$ to determine the specific activity of root respiration. The IRGA-GSC approach is routinely used in our carbon transport studies of plant responses to stress environments.

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