Dissolved CO₂: determination in small sample volumes using the Scholander 0.5-ml analyzer

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D'AOUST, BRIAN G. Dissolved CO₂ determination in small sample volumes using the Scholander 0.5-cc analyzer. J. Appl. Physiol. 30(1): 142-145, 1971.—A simple procedure is described which utilizes an unmodified Scholander 0.5-ml analyzer for extraction and analysis of total dissolved CO₂ in small sample volumes and allows recovery of the analyzed CO₂ for determination of radioactivity. This permits measurement of specific activity on one sample. The method is portable, rapid and useful over a concentration range of 10⁻⁴.

¹³C0₂; CO₂ determination; CO₂ specific activity; CO₂ microanalysis; gasometric analysis; field methods

MICROMETHODS for determining specific activity of dissolved CO₂ (1, 3-5, 9), bicarbonate, or carbonate are limited chiefly by the convenience and sensitivity of the total CO₂ determination, whereas 10⁻⁴ μg of CO₂ is easily measured by liquid scintillation counting. I describe here a simple procedure using an unmodified Scholander 0.5-ml gas analyzer (7) for determining total dissolved CO₂ in small volumes of fluid. All of the sample may then be recovered for counting of radioactivity, thus allowing specific activity determinations on the same sample. As little as 0.020 (±0.004) μmoles and as much as 5.00 (±0.05) μmoles may be analyzed in samples as small as 100 μl. Thus a range in concentration of 10⁴ can be analyzed and specific activities as low as 10⁻³ mc/μmole determined on 0.020 μmoles of CO₂.

PRINCIPLE

The method involves extracting the dissolved CO₂ from a known volume of acid solution into a known volume of CO₂-free air. The CO₂ is then reabsorbed, the volume change recorded, and aliquots of the trapped CO₂ contained in a known volume of solution can be counted in an ion chamber or liquid scintillation counter, the latter providing the most sensitivity. Since both liquid and gas phase volumes are accurately known, empirical corrections for extracted CO₂ may be determined for any particular set of conditions.

PROCEDURE

Familiarity with the apparatus, as detailed in the original paper (7) is assumed in the following procedure, which uses the original reagents. For CO₂ in strongly alkaline samples, as in CO₂ traps, different strength reagents must be used. This was found practical, subject to the requirements of having the water-vapor tension of acid and alkali exactly matched and the need for more time to dissipate the larger amount of heat of neutralization following addition of the alkali. The original reagents (7) are well suited to analysis of physiological strength solutions and natural waters and are used in the method as it is described here.

All precautions described in the original paper are critical in the present application for maximum sensitivity.

Figure I shows the analyzer (labeled as in the original report) with the sample being introduced into the reaction chamber (b) with a 10-μl Hamilton syringe. The life of the Piccolyte coating is only slightly decreased by this procedure. A length of PE-10 catheter tubing can be used to extend the needle tip, if the amount of sample available permits; however, the sample should not be stored exposed to the tubing as considerable CO₂ can be lost.

1) To prepare for sample introduction, the chamber b is rinsed twice with acid rinse to assure that no liquid and gas phase volumes are accurately known, empirical corrections for extracted CO₂ may be determined for any particular set of conditions.

2) The sample is now delivered as shown in Fig. 1 to the bottom of the capillary with the sample being introduced into the reaction chamber. The sample is then drawn into the reaction chamber, and the capillary is adjusted to zero.

3) The acid remaining is removed from a with suction or a syringe and a small drop of acid is then gently removed from a and a small drop of acid is then reabsorbed, the volume change recorded, and free air in chamber b. (There are alternative ways to make sure that CO₂-free air is drawn into chamber b.) The procedure used here was developed in order to leave the apparatus free for conventional gas analysis.

4) Stopcock S-1, filled with a 30-μl disposable glass syringe barrel filled with ascarite or baralyme, is then placed open in the top of a.

5) The top of the acid is then adjusted to the capillary mark, and the micrometer readjusted to zero.

6) With S-2 in position 1, 200 micrometer divisions (40 turns of the micrometer screw) of fresh acid rinse are drawn into the capillary and chamber b.

7) The mercury level is then accurately adjusted to the top of the capillary in a using the micrometer with S-2 in position I.

8) The sample is now delivered as shown in Fig. 1 to the bottom of the capillary with the sample being introduced into the reaction chamber. The sample is then drawn into the reaction chamber, and the capillary is adjusted to zero.

9) To prepare for sample introduction, the chamber b is rinsed twice with acid rinse to assure that no liquid and gas phase volumes are accurately known, empirical corrections for extracted CO₂ may be determined for any particular set of conditions.

10) The sample is then gently removed from a and a small drop of acid is then adjusted to the capillary mark, and the micrometer readjusted to zero.
be taken during this step to prevent acid from flowing past the coating into chamber b, particularly during the next step when introducing the sample. The total volume of solution will be made up of the initial volume of acid, the sample volume, and the alkali. If excess acid is added accidentally during sample addition the correction factor B will differ from that determined from Fig. 2, A and B.

13) S-1 is now replaced on a, closed, and the analyzer is shaken for 2.0 min to assure maximum CO₂ extraction, during which time the reference drop is maintained approximately at the mark with the micrometer screw.

14) After extraction the reference drop is gently lowered to the coating to pick up any drops of acid shaken free during extraction and then readjusted to the mark. This is repeated until the micrometer reading is constant. This reading is then accepted as V₂. A small hand lens is necessary for reading the micrometer with maximum sensitivity. This reading most critically determines the accuracy of the analysis. A dirty capillary will prevent reliable analysis of small quantities of CO₂. The reader is referred to discussion of these factors in the original paper (7).

15) The analyzer is next tilted gently to admit from c into b a volume of CO₂ absorber approximating that of the extracted solution.

16) The analyzer is then shaken for 30 sec, again maintaining the reference drop at the mark using the micrometer screw.

17) Finally, the reference drop is lowered to the coating, slowly raised back to the mark and V₂ read from the micrometer.

When it is desired to recover ¹⁴C CO₂ for determination of radioactivity, it is necessary to measure the total volume of solution in chamber a. This is done as follows. The gas phase is ejected (S-2 in position 1), and the gas/liquid miniscus is set to the mark.

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**FIG. 1. Scholander 0.5-ml gas analyzer, showing introduction of sample with Hamilton syringe placed through acid (400 ml water, 1.00 ml 18.5% H₂SO₄, 72 g Na₂SO₄, 21.0 ml glycerol) in the thermobarometer chamber a, into CO₂-free air-filled chamber b. Black arrow shows tip of needle; white arrow shows 0.5-ml glass syringe barrel filled with baralyme and attached to S-1 for CO₂ absorption during the filling of b; c denotes CO₂ absorber (100 ml H₂O, 11 g KOH, 40 mg K₂Cr₂O₇); 1 is the leveling bulb which is connected to analyzer through S-2. Stopcock S-2 position shown at lower left. Spring-steel wire clips, fashioned so as to continually apply pressure to the rubber stoppers on each side of the glass, are shown in position. These improve accuracy by reducing elasticity. Further details should be sought in ref. 7.**

**FIG. 2. A: Changes in micrometer readings V₁ - V₂ (ordinate) relative to increasing sample size (abscissa) when carried out with CO₂-free samples. This reflects water vapor tension changes caused by diluting the solution with sample. The analysis is improved to the extent that this correction is minimized. B: percentage of CO₂ unextracted E₂ (ordinate) plotted against different gas liquid ratios (abscissa) corresponding to a sample volume range of 1.0 - 500 ml, determined by extracting known amounts of CO₂ for 2.0 min, ejecting the gas phase and counting the radioactivity remaining in the acid. C: time course of extraction of CO₂ in 20-ml sample from acid carried out as described above (ordinate); total counts remaining, abscissa: time). D: total CO₂ in samples analyzed expressed as percent (ordinate) plotted against log of total CO₂ in sample (abscissa). Each value calculated with equation shown in text using corrections B and E₂, taken from graphs A and B. Dotted line shows decrease in standard deviation with increasing total amount of CO₂ analyzed.
REFERENCES of the micrometer during ejection of the liquid phase, and where
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quantity of mercury (near
volume, in micrometer divisions, of an accurately weighed tions (2,
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be taken for determination of radioactivity, and compositions of the sample. The
range of values of E0 shown in Fig. 2B covers a sample volume range of 1 100 µl of samples
whose alkalinity reaches 50 meq liter.

Fig. 2D shows the mean and standard deviation, expressed as percent, of 10 analyses of the increasing amounts of CO2 in samples ranging in concentration from 0.4 to 45.8 ppm. In each case the values have been corrected with values of B and E0 taken from Fig. 2, A and B, respectively. Included in the 10 analyses of each amount of CO2 on the abscissa of Fig. 2D are samples of different volumes and concentrations. For example when total CO2 was 0.02 µmoles, sample volumes used included 0.5 µl of 0.04% NaHCO3 and 50.0 µl of 0.0004% NaHCO3.

The method has been used in studies of CO2 equilibration in the swim bladder of juvenile sunfish, Lepomis megalotis (unpublished data). An additional advantage of the method is that small volumes of whole blood can be directly analyzed as described above, suggesting considerably wider applications. Table 1 compares several analyses of one sample of human heparinized whole blood done with the Van Slyke and the Scholander 0.5 ml analyzer.

Reproducibility is lowered by blood, chiefly due to small amounts of protein sticking to the capillary and changing the distribution of forces on the reference drop. This is largely eliminated by thorough rinsing between analyses. The blood is well broken up during extraction, and proteins deuterated during the acid extraction appear to become more soluble and less adhesive on addition of the alkali.

Obvious advantages of the method, namely, the portability of the apparatus, the small sample size required, the range of concentrations which may be analyzed with no need for modifications (2, 8, 10), make it a useful field method. For example, 20 µl of seawater have enough CO2 to analyze at the lower limit of sensitivity (approximately 0.01 µmoles). This will allow reliable measurements of metabolic 11C02 to be made with small aquatic organisms in small volumes of seawater or larger volumes of freshwater (6).

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Contribution from the Bureau of Medicine and Surgery, Navy Department, Research Task NR003-004-0115. The opinions expressed here are those of the author and do not necessarily reflect the views of the Navy Department or the naval service at large.

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TABLE 1. Total CO2 in whole blood

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<th>Blood vol, ml</th>
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<td>1.00</td>
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<tr>
<td>1.00</td>
<td>22.0</td>
<td>10.8</td>
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μmoles total CO2 (STP) = \( \frac{(V_a - V_b) \times F}{100 - E_0} \times \frac{273}{273 + 4°C} \)

where \( V_a \) and \( V_b \) are the initial and final readings of the micrometer before and after CO2 absorption, B is a correction, empirically determined, which compensates for differences in the vapor tension of the acid/sample solution and that of the alkali according to the volume of sample analyzed (Fig. 24). F is a conversion factor (which is constant for any one apparatus and temperature) whose units are (μmoles)/(micrometer div) at STP, and \( E_0 \) expresses the fraction of CO2 unextracted at equilibrium (Fig. 2B). The value F is determined by measuring the volume, in micrometer divisions, of an accurately weighed quantity of mercury (near 0.500 g) delivered into b with a Hamilton syringe. Thus

\[ F_{eq} = \frac{22.26}{\text{(micrometer div)}} \times \frac{\text{density Hg at } 1°C}{\text{mg}} \]

A value of 0.0167 was determined in the present analyzer. Corrected for a temperature of 23 C, F decreased to 0.0154 μmoles/micrometer division.

The liquid volume in microliters is determined as \( V_a - V_b \). L, where \( V_a \) and \( V_b \) are the initial and final readings, respectively, of the micrometer during ejection of the liquid phase, and L is the volume calibration factor of the system (in units of mm3/ div-1 wt Hg/(density Hg × micrometer divisions)). Knowing the volume of the liquid prior to absorption of CO2, the gas/liquid volume ratio \( V_b/V_a \) is calculated and the percent of CO2 unextracted, E0, read off the ordinate in Fig. 2B. This curve is determined using the above procedure on samples, a known amount of radioactive 11CO2, rapidly ejecting the gas phase and determining the residual radioactivity after a 2.0 min extraction (Fig. 2C). The maximum correction \( E_0 \) found with a 100.0 µl sample of 0.0438 µM NaHCO3 was 5.3%. This correction should be determined on solutions approximating the ionic strength and alkalinity of the sample to be analyzed. The distribution law holds that with temperature, and hence gas and liquid phases is independent of pressure. However, the CO2 solubility of the liquid phase will change with the volume and composition of the sample. The range of values of \( E_0 \) shown in Fig. 2B covers a sample volume range of 1 100 µl of samples whose alkalinity reaches 50 meq liter.

Figure 2D shows the mean and standard deviation, expressed as percent, of 10 analyses of increasing amounts of CO2 in samples ranging in concentration from 0.4 to 45.8 ppm. In each case the values have been corrected with values of B and \( E_0 \), taken from Fig. 2A and B, respectively. Included in the 10 analyses of each amount of CO2 on the abscissa of Fig. 2D are samples of different volumes and concentrations. For example when total CO2 was 0.02 µmoles, sample volumes used included 0.5 µl of 0.04% NaHCO3 and 50.0 µl of 0.0004% NaHCO3.

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Dissolved CO₂ Determinations in Small Samples


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