

Short communication
Technique to measure CO₂ mixing ratio in small flasks
with a bellows/IRGA system

D.R. Bowling*, C.S. Cook, J.R. Ehleringer

*Stable Isotope Ratio Facility for Environmental Research, Department of Biology,
University of Utah, 257 S. 1400 E., Salt Lake City, UT 84112-0820, USA*

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Abstract

We developed a laboratory method to obtain high precision CO₂ mixing ratios in air samples contained in 100 ml glass flasks, which involves extracting air from the flask into a pre-evacuated manifold containing a variable-volume metal bellows. Compressed air from the bellows is then routed to a non-dispersive infrared gas analyzer (IRGA). Tests indicate that this configuration has an overall accuracy of ± 0.3 and a precision of $\pm 0.2 \mu\text{mol mol}^{-1}$ and will work with flask volumes as low as 64 ml air (STP). When combined with continuous-flow mass spectrometry approaches, both atmospheric CO₂ mixing ratios and isotope ratio analyses can now be routinely analyzed with small flasks, enabling more extensive sampling of CO₂ exchange processes between the biosphere and the atmosphere. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

There is considerable interest at present in understanding CO₂ exchange between terrestrial ecosystems and the atmosphere, with scores of sites around the world routinely monitoring net ecosystem carbon exchange. Of central importance to such studies are accurate and precise measurements of CO₂ mixing ratio¹ ([CO₂]) in air. Commercial infrared gas analyzers (IRGAs) are available from a variety of manufacturers and can provide excellent results. However, accurate control of the pressure, temperature, and humidity en-

vironment of these instruments, as well as the careful maintenance of stable calibration gases are required for accuracies of $< 0.5 \mu\text{mol mol}^{-1}$. Such constraints can be difficult to overcome at remote field sites lacking electrical power or buildings.

The addition of stable isotope analyses of atmospheric CO₂ to atmospheric and flux monitoring stations is beginning to provide new insight into carbon cycle processes (Ciais et al., 1995; Flanagan and Ehleringer, 1998). Not too long ago, large air samples (2 l) were required for high precision ($< 0.05\%$) measurements of CO₂ isotopic composition (e.g., Flanagan et al., 1996; Troler et al., 1996). Although the NOAA Global Air Sampling Network routinely sends 2.5 l flasks around the world, large flasks are cumbersome for intensive field studies, especially where hundreds may be required.

* Corresponding author. Tel.: +1-801-581-8917;
fax: +1-801-581-4665.

E-mail address: bowling@biology.utah.edu (D.R. Bowling).

¹ We use “mixing ratio” in this paper to denote mixing ratio by mole fraction of CO₂ in air in units of $\mu\text{mol mol}^{-1}$.

Recent developments in continuous-flow isotope ratio mass spectrometry now allow high precision isotope ratio measurements of CO_2 to be made on 50–500 μl of air (Brand, 1996; Ehleringer and Cook, 1998; Ferretti et al., 2000). Earlier, we developed a routine procedure to determine CO_2 isotopic composition in 100 ml glass flasks, which increases by an order of magnitude the number of flasks that can be managed at a remote field site relative to the larger size (Ehleringer and Cook, 1998). Until now, our laboratory has been measuring $[\text{CO}_2]$ during sample collection in the field with a portable photosynthesis system located downstream of the flask. This can be a limitation because (1) pressure effects associated with different sampling line lengths and flow rates can substantially affect measured $[\text{CO}_2]$ and (2) highly accurate calibrations are difficult to be maintained under field conditions where environmental conditions vary.

We describe here a laboratory based method to measure $[\text{CO}_2]$ in small flask samples using an IRGA coupled to a variable-volume bellows.

2. System description

The general principle behind the $[\text{CO}_2]$ measurement is to (1) extract as much gas as possible from

the sample flask, (2) pressurize the extracted gas above local atmospheric pressure, and (3) use this high pressure to flush the gas through the sample cell of a CO_2 analyzer. A similar apparatus with a variable volume is used in the NOAA/CMDL Global Air Sampling Network (Komhyr et al., 1983; Thoning et al., 1995). Our system was designed with several constraints in mind. The volume of the manifold with the bellows expanded was maximized to extract as large a sample as possible and the compressed volume was minimized to allow maximum compression pressure. The connection volume between the manifold and the IRGA was kept small for maximum flushing efficiency of the sample cell.

The apparatus is diagrammed in Fig. 1. All components of the system are stainless steel or aluminum. The stainless steel bellows (MEW4974001E, Kurt J. Lesker, Pittsburgh, PA) has an internal volume of 285 cm^3 when open and 36 cm^3 when closed, the latter achieved by adding a machined steel disk to the inside of the bellows. The top and bottom of the bellows are each welded to a steel annulus and bolted to an aluminum disk, and sealed with O-rings. The disks move up and down on aluminum rods, which constrain movement to one spatial dimension. Bellows movement is controlled by a pneumatic air cylinder (2A713, W.W. Grainger, Lake Forest, IL). Manifold

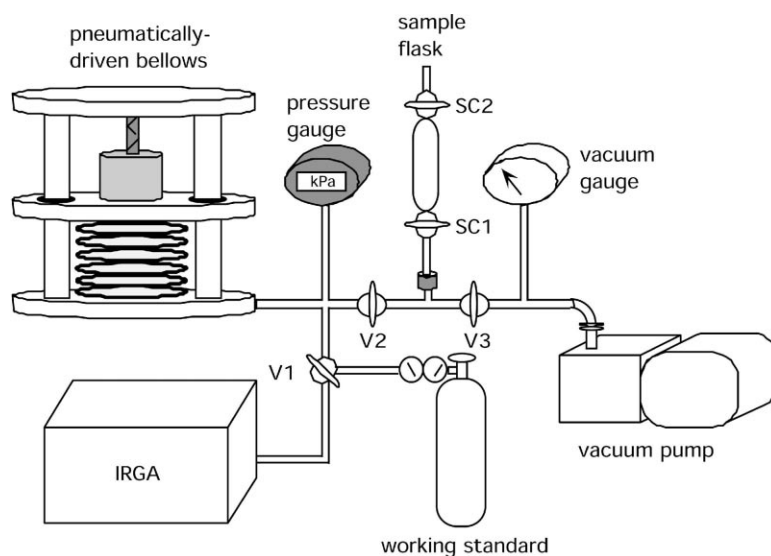


Fig. 1. A diagram of the system.

components and connections are stainless steel, and steel rods are inserted into the manifold to reduce its internal volume. The volumes of the manifold are small: 9.3 cm³ between valve 1 and valve 2 (V1 and V2) excluding the bellows, and 4.3 cm³ between V2, V3, and SC1.

The CO₂ analyzer used is an LI-6262 IRGA (LI-COR, Lincoln, NE) operated in absolute mode. The chopper assembly is chemically scrubbed of CO₂ and H₂O in a closed path, and the reference cell is continuously purged with chemically scrubbed N₂. The sample cell inlet is connected to the gas manifold, and the sample outlet is open (through a length of tubing) to the atmosphere. The sample cell inlet on the LI-6262 was replaced with $\frac{1}{16}$ in. stainless steel tubing to (1) slow the flow when routing gas from the manifold to the IRGA to assure more effective sample cell flushing and (2) minimize the volume (0.4 cm³) between the manifold (V1) and the sample cell.

A measurement cycle proceeds as follows. The sample cell is first flushed with a working CO₂ standard (through V1) that is near the contemporary atmospheric mixing ratio. A sample flask (34-5671, Kontes Glass, Vineland, NJ, see also Ehleringer and Cook, 1998) is attached to the manifold. The flask stopcocks (SC1 and SC2) remain closed initially, V1 is closed, the bellows is compressed, and V2 and V3 are opened. The manifold is evacuated to <10 Pa with a vacuum pump (E2M5, Edwards High Vacuum, Wilmington, MA) and monitored by a thermocouple vacuum gauge (type 0531, Varian Vacuum Technologies, Lexington, MA). The bellows is expanded and V3 is closed to the vacuum. SC1 is then opened and sample gas flows into the manifold. The manifold pressure at this point is 22–25 kPa, depending on initial flask pressure (pressure is monitored with a steel diaphragm sensor, P-68801-53, Cole-Parmer Instrument, Vernon Hills, IL). V2 is then closed to exclude the volume of the flask neck and the bellows is compressed. This increases the manifold pressure by a factor of roughly 6.9 to 154–168 kPa, significantly above ambient pressure (86 kPa in our laboratory). We will refer to the pressure at this point as the “compressed manifold pressure”. V1 is then opened to let the sample flow through the IRGA. Within 5 s the IRGA pressure stabilizes and the CO₂ reading is recorded.

The IRGA is calibrated with chemically scrubbed N₂ (soda lime and magnesium perchlorate, to zero)

and a WMO-traceable CO₂-in-air mixture near ambient [CO₂]. Calibration gases are introduced via V1. All calibrations and measurements are made with the sample cell at ambient pressure (no flow and vented to the atmosphere). Water vapor is removed from all samples during collection with magnesium perchlorate.

3. System performance

Several tests were performed to assess the accuracy and precision of the system. The sample cell volume of the LI-6262 CO₂ analyzer is only 11.9 cm³, but its rectangular shape is not well suited to efficient gas turnover with small sample volumes. Our system design assures several turnovers of air within the IRGA sample cell volume to avoid inadequate IRGA flushing, which might lead to a memory effect from one sample to the next. To test for a memory effect, 10 sample flasks were filled in series with air of known [CO₂] (409.7 μmol mol⁻¹). Before each measurement, the IRGA sample cell was flushed with CO₂-free air, providing a large difference between initial and final [CO₂] values. Measured [CO₂] in the flasks was 409.2 ± 0.2 μmol mol⁻¹ (mean ± 1 S.D., *n* = 10), suggesting that there is indeed a small amount of residual air that remains in the cell between measurements (99.88% air turnover). For this reason, we flush the sample cell with a working standard (near 400 μmol mol⁻¹) before each measurement and then the memory effect is minimal.

The volume of gas that is flushed through the IRGA is an important parameter. A typical sample size is 100 ml at field pressure, which varies with altitude. We remove several milliliters from each sample during isotope ratio measurements prior to using the bellows system. The memory-effect test above was performed at compressed manifold pressures of 173 ± 9 kPa (*n* = 10), which corresponded to a flushing volume at ambient laboratory pressure of 90 cm³.

To assess just how high the final manifold pressure must be to achieve adequate cell flushing, we repeatedly measured the same sample in a large (1.7 l) flask, in which the CO₂ value was 498.4 μmol mol⁻¹ and the working standard was 409.7 μmol mol⁻¹ (Fig. 2). We were able to obtain precise estimates of the sample CO₂ value over a wide range of compressed manifold

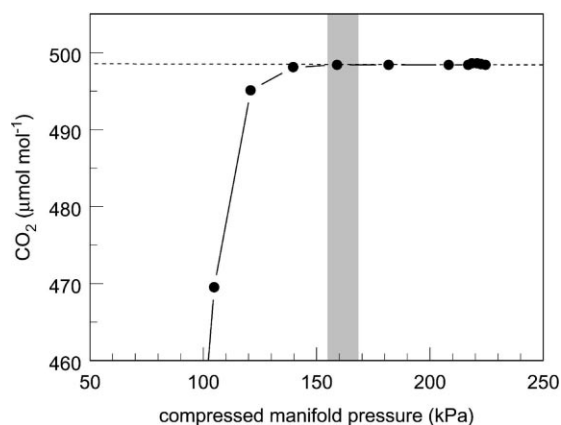


Fig. 2. Measured CO₂ (solid circles) versus compressed manifold pressure during repeated measurements of the same 1.7 l flask. The dashed line represents the actual CO₂ mixing ratio of the gas (498.4 μmol mol⁻¹) and the shaded box indicates the range of compressed manifold pressures associated with typical field-collected 100 ml flasks, which were sampled at elevations ranging from 0 to 1000 m.

pressures. Only at the lowest compressed manifold pressures (<140 kPa) was there insufficient turnover of air within the IRGA sample cell. The measured CO₂ is very consistent (the standard deviation of the first nine samples was 0.1 μmol mol⁻¹) as long as the compressed manifold pressure stays above 140 kPa (a flushing volume of about 74 cm³ in our laboratory (1450 m) or 57 cm³ at STP). Below this pressure, there is insufficient gas in the manifold to adequately flush the sample cell and the measured CO₂ approaches the working gas value.

To assess the repeatability of the CO₂ measurements, we filled and measured five sets of 10 flasks in series with [CO₂] ranging from 345.5 to 498.4 μmol mol⁻¹. Three different operators analyzed the flasks with a random selection of flasks assigned to each. The standard deviation of the individual measurements from each 10-flask mean was 0.17 μmol mol⁻¹ ($n = 50$) and the range was -0.46 to 0.31 μmol mol⁻¹. Thus, we estimate our analytical precision as 0.2 μmol mol⁻¹, which is near the noise limit of the LI-COR CO₂ analyzer.

We assessed the accuracy of the bellows/IRGA system by measuring flasks filled from calibration tanks. Fifty flasks were filled individually from each of five WMO-traceable tanks covering the typical range of

atmospheric mixing ratios (345.5–498.4 μmol mol⁻¹). Flasks were unlabeled and measured in random order by two different researchers, with a working standard of 409.7 μmol mol⁻¹. The measured mixing ratios of the 50 flasks were compared to their respective tank values and the standard deviation of this difference was 0.30 μmol mol⁻¹ with a range of -0.7 to 0.7 μmol mol⁻¹ ($n = 50$). Thus, we report 0.3 μmol mol⁻¹ as the absolute accuracy of our system, which is better than the manufacturer's stated accuracy for the LI-6262 analyzer (± 1 μmol mol⁻¹ at 350 μmol mol⁻¹).

Our samples are typically analyzed 1–2 weeks after field collection. In laboratory tests, we have observed a flask memory effect when very high CO₂ mixing ratios (>10,000 μmol mol⁻¹) are followed by samples at ambient [CO₂]. The flasks have Teflon stopcocks, which could alter [CO₂] over time, especially considering the small volume of the flasks relative to stopcock surface area. The temporal stability of [CO₂] in the flasks was examined by filling 63 flasks with the same gas (399.0 μmol mol⁻¹), and analyzing them in groups of 5–7 flasks each, after allowing the air samples to age between 0 and 32 days. Results did not differ between the groups and the overall standard deviation of the measured [CO₂] was 0.22 μmol mol⁻¹ ($n = 63$). Stability of carbon isotope ratios over a similar time frame (28 days) was confirmed with additional tests (data not shown).

The inherent nonlinearity of the IRGA response will contribute to measurement precision. Our results could potentially be improved by using a calibration strategy that includes several frequently measured standards *within* the range of the expected measurements combined with a second-order polynomial fit (Komhyr et al., 1983; Bakwin et al., 1995) instead of the standard two-point zero-and-span calibration.

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