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An automated system for stable isotope and concentration analyses of CO_2 from small atmospheric samples

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We have developed an automated, continuous-flow isotope ratio mass spectrometry (CF-IRMS) system for the analysis of $\delta^{13}C$, $\delta^{18}O$, and CO_2 concentration ($\mu mol\, mol^{-1}$) ($[CO_2]$) from 2 mL of atmospheric air. Two replicate 1 mL aliquots of atmospheric air are sequentially sampled from fifteen 100 mL flasks. The atmospheric sample is inserted into a helium stream and sent through a gas chromatograph for separation of the gases and subsequent IRMS analysis. Two $\delta^{13}C$ and $\delta^{18}O$ standards and five $[CO_2]$ standards are run with each set of fifteen samples. We obtained a precision of 0.06‰, 0.11‰, and 0.48 $\mu mol\, mol^{-1}$ for $\delta^{13}C$, $\delta^{18}O$, and $[CO_2]$, respectively, by analyzing fifty 100 mL samples filled from five cylinders with a $[CO_2]$ range of 275 $\mu mol\, mol^{-1}$. Accuracy was determined by comparison with established methods (dual-inlet IRMS, and nondispersive infrared gas analysis) and found to have a mean offset of 0.00‰, -0.09‰, and -0.26 $\mu mol\, mol^{-1}$ for $\delta^{13}C$ and $\delta^{18}O$, and $[CO_2]$, respectively. Copyright © 2005 John Wiley & Sons, Ltd.

Stable isotope ratio analyses of both organic matter and of trace gases have become integral components of carbon cycle research.[1–5] Here isotopic and concentration analyses of CO_2 in air samples provide important insights into the factors influencing movement of carbon from one pool to another. As a trace gas, atmospheric CO_2 had posed an analytical challenge simply because of the large sample volumes required for dual-inlet isotope ratio mass spectrometry (DI-IRMS) approaches (see, e.g., Ref. 1). The development of continuous-flow isotope ratio mass spectrometry (CF-IRMS) made possible an alternative analytical approach with the potential for a much smaller atmospheric sample volume required for a CO_2 isotope ratio measurement, while maintaining reasonable precision and accuracy. The CF-IRMS approach also made possible increased sample throughput, a limiting factor for many atmospheric studies.

Several CF-IRMS techniques for isotope ratio analyses of atmospheric CO_2 samples have been developed.[6–9] Each of these approaches involve an online gas chromatographic (GC) separation of CO_2 and N_2O. One major distinction among these approaches is the use either of glass flasks with stopcocks allowing for long-term storage of the atmospheric sample or of septum-capped vials,[9] and improvements are being made to prolong the storage capacity of these vials.[10] The capacity to provide a measurement of CO_2 concentration ($\mu mol\, mol^{-1}$), hereafter $[CO_2]$, simultaneously with the isotope ratio measurements is an important feature. Other considerations for CO_2-in-air CF-IRMS analyses include use of cryogen, analysis time, precision, and accuracy.

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Here we describe a new automated system that provides both high-precision isotope ratio measurements on CO_2 in atmospheric samples as well as $[CO_2]$. The measurement system uses two 1 mL aliquots extracted from 100 mL glass flasks (with stopcocks), does not require a cryogen, has a complete analysis time of approximately 17 min/sample, and can be operated continuously. Hence, approximately 50 atmospheric samples can be analyzed per day (including standards) for $\delta^{13}C$, $\delta^{18}O$, and $[CO_2]$.

EXPERIMENTAL

System design

Figure 1 illustrates the plumbing design of the automated system. The system contains air-actuated valves (SS-4BK-TW-1C, Swagelok Co., Cleveland, OH, USA) for manifold control. Connections are made with stainless steel micro-fit weld fittings (6LV-4MW-*, Swagelok Co.) to minimize volume. A 50 mL stainless steel electrically driven variable volume bellows (MEW3502001E, Kurt J. Lesker Company, Clairton, PA, USA) is used to pressurize the sample loop portion of the manifold as needed. A pressure gauge (68801-57, Cole Parmer Instrument Co., Vernon Hills, IL, USA) and a vacuum gauge (0531, Varian Inc., Palo Alto, CA, USA) are used to monitor manifold conditions. A vacuum pump (E 2M1.5, BOC Edwards, Wilmington, MA, USA) evacuates the manifold initially, and following each sample. A two-position, six-port valve (ETC6UWE, Valco Instruments Co., Inc. (VICI), Houston, TX, USA) is used to load and purge a 1 mL stainless steel sample loop (SL1KCUW, VICI). The automated system is designed to service 20 100 mL flasks (34-5671, Custom Glass Shop at Kontes, Vineland, NJ, USA), of which five are $[CO_2]$ standards, and also two 2 L isotope standard flasks

(65-3210, Kontes). Sample and standard flasks are secured with vacuum compression fittings (Ultra-Torr, SS-*-UT-*, Swagelok Co.). Air-actuated valves throughout the manifold are controlled by solenoids (EV-3M-12, Clippard, Cincinnati, OH, USA). The automated system is controlled by a datalogger (CR23x, Campbell, Logan, UT, USA) which in turn is signaled by an air-actuated contact closure switch for IRMS/automated system communication (PE-1/8-1N, Festo, Hauppauge, NY, USA). Aliquots of air flow through a GC column (Carboxen 1010 Plot, fused-silica capillary column, 30 m × 0.32 mm, Supelco, Bellefonte, PA, USA) for gas species separation. Sample gas then flows through an open-split interface with a backflush modification (GP-Interface, Thermo-Finnigan, Bremen, Germany) and then into a Delta Plus XL with universal triple collector (Thermo-Finnigan) for isotope ratio analysis and peak area quantification.

System operation

Samples and standards are loaded on the system and the entire manifold, including the airspace up to the flask stopcocks, is evacuated. Users initiate the IRMS instrument after the manifold is evacuated and the instrument then provides a start signal to the datalogger to begin processing sample air from a flask. A sample with a pressure of approximately 90 kPa is then expanded into the manifold and bellows (Fig. 1) yielding approximately 35 kPa inside the bellows. The bellows close until the pressure in a reduced-volume portion of the manifold (shown as solid tubing in Fig. 1) reaches 107 kPa at which point the sample is expanded into an evacuated 1 mL sample loop, leaving ∼100 kPa in the bellows. Excess pressure in the sample loop is vented to equilibrate with atmospheric pressure (∼90 kPa in Salt Lake City, UT)

and then the two-position valve rotates to introduce the first of two aliquots into a helium stream with a 2.0 mL min^{-1} flow rate. The sample passes to the gas chromatograph held at 140°C for gas species separation, the open-split interface, and to the mass spectrometer for analysis. Once the first aliquot of sample air has arrived at the source (∼375 s), the bellows compress again to pressurize the manifold to 107 kPa. The sample air contained in the bellows is, again, expanded into the evacuated 1 mL sample loop, allowing excess pressure to vent, and then introduced into the helium stream for separation and analysis as in the first aliquot.

A time-series trace of a typical single-flask run is illustrated in Fig. 2. Instrument working reference gas (100% CO_2, $\delta^{13}C = -11.21$‰, $\delta^{18}O = 20.63$‰) is introduced into the IRMS instrument through the modified GP interface and measured twice before the first aliquot sample peak, between the two aliquot sample peaks, and after the second aliquot sample peak. The second and fourth instrument reference CO_2 peaks are used as formal standard peaks for delta calculations while the first and third instrument reference CO_2 peaks are used to keep the source conditioned. The Delta Plus XL tuned for signal/isotope ratio linearity yields an amplitude of 400–700 mV on cup 1 (mass 44) for [CO_2] corresponding to 300–600 μmol mol^{-1}. The sample peak width remains constant due to the 1 mL sample loop and the peak areas for similar [CO_2] range from 15–27 Vs.

System performance

We tested our system for precision and accuracy in $\delta^{13}C$, $\delta^{18}O$, and [CO_2] of CO_2 as compared with an independent method of measuring isotopes (DI-IRMS) and, separately, concentration (nondispersive infrared gas analysis (NDIR)).
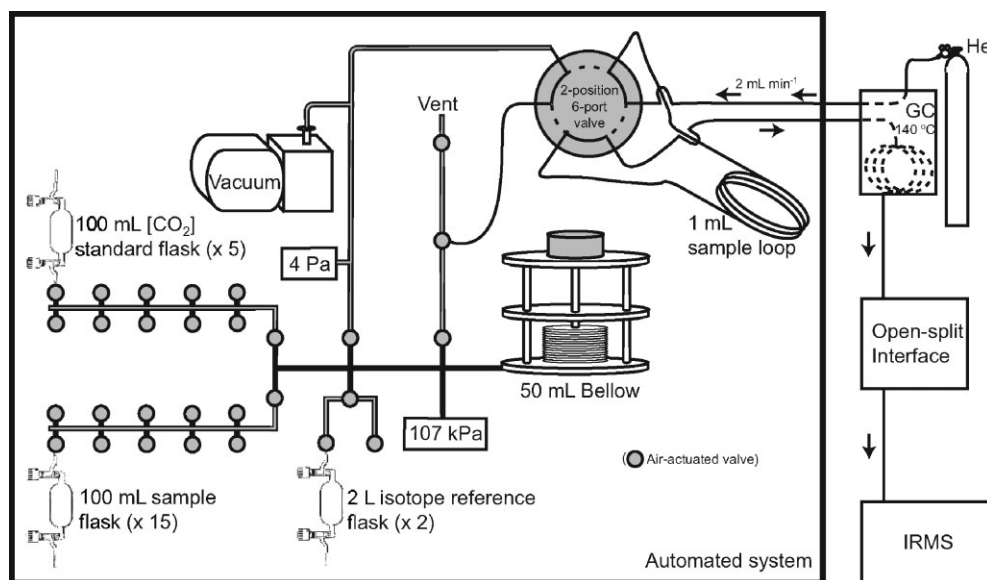

Figure 1. Schematic of the automated system. Air is expanded from a flask into an evacuated manifold, including a variable volume bellows. The bellows volume is isolated from the greater manifold and sample air is pressurized to 107 kPa by bellows compression. Sample air is expanded into an evacuated sample loop and excess air pressure in the sample loop vents to equilibrate with atmospheric pressure. The two-position six-port valve rotates and introduces the first of two aliquots into a 2 mL min^{-1} helium stream, moving the sample to a 140°C gas chromatograph for gas species separation and then to the open-split interface and analysis by IRMS.
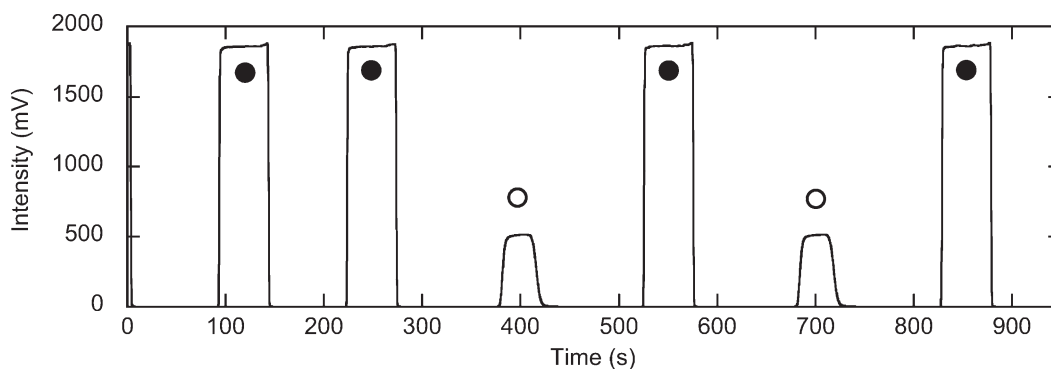
Figure 2. A typical run of one flask showing a time-series trace of four instrument-reference gas (100% CO$_2$) peaks (●) introduced from the GP interface and two replicate sample peaks (○) introduced from the automated system.

Five CO$_2$-in-air test cylinders were prepared with varying [CO$_2$]. These cylinders were measured for [CO$_2$] using NDIR (Li-7000, LiCor, Lincoln, NE, USA). The infrared gas analyzer was calibrated using five [CO$_2$] calibration cylinders that were measured for concentration by NOAA/CMDL (CC153217, CC153264, CC163396, CC163398, CC163466). The concentrations (and standard deviations of three measurements) of the five [CO$_2$] calibration cylinders are: 328.01 (0.02), 360.87 (0.01), 427.20 (0.04), 509.63 (0.02), and 602.26 (0.08) μmol mol^{-1}. The five test cylinders were measured for $\delta^{13}C$ and $\delta^{18}O$ by DI-IRMS (Delta Plus Advantage, Thermo-Finnigan). A vacuum line was used to cryogenically extract CO$_2$ from purged 2 L flasks to 6 mm Pyrex tubes to provide CO$_2$ for DI-IRMS analysis. Ten 2 L flasks were purged using each of the five test cylinders. The presence of N$_2$O was accounted for by measuring the concentration, ionization efficiency, and isotope ratios and corrected for accordingly.[11,12] Extracted CO$_2$ was measured against a 100% CO$_2$ standard (OzTech) with $\delta^{13}C = -3.64$‰ (VPDB), and $\delta^{18}O = 24.97$‰ (VSMOW), which was created and measured by OzTech Trading Corp. (Dallas, TX, USA) against a suite of international standards including NBS 18, 19, 20, 22, 28, and 30 (K. Ferguson, C. Douthitt, personal communication, 2004), and subsequently verified at our laboratory with NBS 21 (Graphite) for $\delta^{13}C$.

Ten 100 mL flasks were purged with air from each of the five test cylinders for analysis by the automated system.

These 50 sample flasks were analyzed over the course of four runs. Five [CO$_2$] standard 100 mL flasks were purged from each of the NOAA/CMDL calibrated cylinders and run with 15 of the sample flasks. Two 2 L flasks were purged with cylinders used as isotope standards. These isotope standards have an approximate [CO$_2$] of (1) 395 μmol mol^{-1} and (2) 480 μmol mol^{-1}. (Note these isotope standards are processed through the automated system as atmospheric samples as opposed to the instrument reference gas (100% CO$_2$) that is introduced via the GP interface.) The CO$_2$ in these cylinders was extracted and measured by DI-IRMS against OzTech and have values (and standard deviations) as follows: (1) $\delta^{13}C = -9.41$ (0.03) VPDB; $\delta^{18}O = 39.53$ (0.06) VSMOW and (2) $\delta^{13}C = -16.00$ (0.03) VPDB; $\delta^{18}O = 27.24$ (0.04) VSMOW. Both isotope standards are sampled at the beginning of a bank of 20 flasks, then again every five samples, and finally at the end of the bank of 20 flasks.

Once the 15 samples, five [CO$_2$] standards, and repeated measurements of the isotope standards had been run (17 min × (15 + 5 + 10) = 8.5 h), three corrections were applied. From the repeated measurements of the isotope standards, a linear measured-to-actual correction for $\delta^{13}C$ and $\delta^{18}O$ was applied to each aliquot and the two aliquots per flask were averaged to yield final $\delta^{13}C$ and $\delta^{18}O$ results. Total peak area was used to obtain [CO$_2$] for each sample. The peak area of the instrument working reference gas was used to normalize each aliquot peak area for drift in instrument

Table 1. Accuracy and precision of the automated system with the five test cylinders. Precision is calculated as the standard deviation of the samples analyzed on the automated system (n = 10). Accuracy is calculated as the difference between the mean DI-IRMS/NDIR measurements (n = 10) and the mean of the automated system measurements (n = 10). Overall is the precision or accuracy for all five cylinders, combined

Cylinder	Cylinder values from dual inlet or infrared gas analysis			Dual inlet or infrared gas analyzer precision			Automated system precision			Automated system accuracy		
	$\delta^{13}C$	$\delta^{18}O$	[CO$_2$]	$\delta^{13}C$	$\delta^{18}O$	[CO$_2$]	$\delta^{13}C$	$\delta^{18}O$	[CO$_2$]	$\delta^{13}C$	$\delta^{18}O$	[CO$_2$]
A	−8.34	40.52	328.31	0.03	0.07	0.05	0.08	0.12	0.61	0.06	0.04	−0.38
B	−8.32	40.49	361.69	0.03	0.16	0.06	0.08	0.15	0.54	0.05	0.00	−0.35
C	−9.37	37.53	425.76	0.02	0.26	0.06	0.05	0.07	0.33	−0.05	−0.11	−0.19
D	−10.18	34.83	509.31	0.02	0.24	0.05	0.02	0.10	0.40	−0.03	0.01	−0.18
E	−9.29	32.38	603.92	0.03	0.10	0.06	0.04	0.08	0.51	−0.04	−0.36	−0.18
Overall	**n/a**	**n/a**	**n/a**	**0.03**	**0.17**	**0.05**	**0.06**	**0.11**	**0.48**	**0.00**	**−0.09**	**−0.26**

sensitivity. The aliquot peak areas per flask were averaged. A linear peak area-to-[CO$_2$] correction curve was generated using the five [CO$_2$] standards and applied to each sample to yield final [CO$_2$] results. This process was completed separately for each of the four runs needed to analyze the 50 sample flasks included in this analysis.

RESULTS

Table 1 shows the precision and accuracy of the automated system measured as the standard deviation (SD) of all measurements and the difference of the automated system measurement mean from the DI-IRMS and NDIR measurement mean, respectively. The overall δ^{13}C and δ^{18}O precisions (SD of respective test cylinder) for 50 DI-IRMS measurements of extracted 2 L flasks were 0.03‰ and 0.17‰, respectively. The overall automated system precisions for δ^{13}C and δ^{18}O of 50 100 mL flasks were 0.06‰ and 0.11‰, respectively. The overall [CO$_2$] precision for 50 NDIR measurements was 0.05 µmol mol^{-1} while that of the automated system was 0.48 µmol mol^{-1}. The overall accuracy (difference from DI-IRMS or NDIR and the automated system) is 0.00‰, −0.09‰, and −0.26 µmol mol^{-1} for δ^{13}C, δ^{18}O, and [CO$_2$], respectively.

DISCUSSION

We present an automated system for CF-IRMS analysis of δ^{13}C, δ^{18}O, and [CO$_2$] from CO$_2$ in 100 mL air samples. A significant advantage of this system is that it does not require a cryogen, but rather uses only gas chromatography for separation of gas species. This cuts down appreciably on normal operating costs and facilitates unattended operation. The analysis cycle time was approximately 17 min/sample and 8.5 h for an entire bank of 15 samples, five concentration standards and five repeated measurements from two isotope standards. The isotope ratio precision approached that of DI-IRMS. The concentration precision of 0.48 µmol mol^{-1} is acceptable for many carbon cycle applications, especially if the small sample size allows for replicated field sampling to obtain a better appreciation of the atmospheric variability at a field site over time. The noise in the automated system (precision) was greater than the difference from DI-IRMS and NDIR established methodology (accuracy).

Given the scale of the 0.05‰ minute-to-minute variability in the isotope ratios of CO$_2$ under terrestrial field conditions,[13,14] the measurement system described here is more than sufficient for high-precision atmospheric sampling of isotopes in CO$_2$ in terrestrial ecosystems. Future development and improvement could be focused on increasing the signal-to-noise ratio for increased [CO$_2$] precision. This might

be accomplished by an increase in ionization efficiency from the current 1000:1 ratio. Alternatively, a thermal conductivity detector (TCD) could be added downstream of the gas chromatograph for measurement of CO$_2$ peak area. Increasing the volume of the sample loop with a concomitant increase in GC column length might also increase the CO$_2$ signal, but would require greater time per sample. Small sample volume allows for additional applications of this approach such as sampling soil air volumes.

The automated system used in conjunction with a field-automated sampler[15] provides researchers with the ability to collect small-volume air samples from remote locations at a reasonable frequency and analyze them in a timely manner for ecophysiological and micrometeorological applications.[16,17]

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