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Article

Enhanced Stability of Stable Isotopic Gases

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ABSTRACT: Pure gases and mixtures containing stable isotopes are used in a wide variety of applications including health care, food authentication, geochemistry, and environmental monitoring. It is therefore important to understand the role of moisture, which is one of the most critical impurities in compressed gas mixtures and pure gases, in their stability. Gaseous carbon dioxide (CO_2) was used as a proxy for the evaluation of the effects of its isotopic composition, when in contact with moisture throughout the depletion of a cylinder's contents, as well as pressure regulation and long-term stability. To accentuate the detrimental effects of moisture on CO₂ isotopic stability, enriched ¹⁸O-water was added to natural-abundance, gaseous carbon dioxide. The $\delta^{18}O-CO_2$ changed from -23.16% vs Vienna Pee Dee Belemnite (VPDB) to +109% vs VPDB. It was further demonstrated that with appropriate cylinder preparation to reduce residual moisture, source material purity with low moisture content, and pressure regulation (from 57.0 down to 0.5 bar), both δ^{13} C and δ^{18} O remained consistent within ±0.04 and ±0.06‰, respectively, throughout the entire cylinder contents. Pressure reduction using a dual-stage regulator yielded statistically consistent results at the 99% confidence level from delivered pressures of 0.1-0.8 bar. Furthermore, the isotopic values



remained consistent during a 1 year shelf-life study, illustrating the ability to utilize and regulate pressurized gases as working reference standard gases.

■ INTRODUCTION

Stable isotopes have been an integral part of the toolbox of many branches of science and technology and have seen an increase in areas such as food authentication and environmental research.¹ At the heart of the toolbox is the requirement for stable calibration and working gas mixtures used for analyzer calibration. Calibration gases are often used as working gases or calibration gases for stable isotope measurements. However, these calibration and working gases are sometimes thought of as unstable, and the longer the gas remains within a cylinder, the larger the discrepancy will be with the original isotopic values.² Furthermore, it is believed that as the cylinder is depleted or the pressure is regulated, the isotopic ratio of the gas will change (due to fractionation).^{3,4}

Examining the catalogs of industrial and specialty gas manufacturers, it is evident that there is a wide range of specifications available for all pure gases. Stated purities range from 99.9 to 99.999%. At a purity level of 99.999%, no more than 10 ppm total specified impurities will be present in the gas, whereas 99.9% contains no more than 1000 ppm total specified impurities. These impurities can include moisture, hydrocarbons, carbon dioxide, carbon monoxide, oxygen, and other impurities. The cylinder preparation, which includes the use of high-purity cylinders, vacuum baking, and measurement of trace impurities are not as rigorous for industrial quality gases or those with lower purity specifications.

In a compressed gas cylinder, the molecules of each component within the gas mixture are constantly in motion according to the kinetic theory of gases. This helps maintain

the homogeneity of the mixture. However, this is also one of the primary reasons that reactive components in a mixture decompose.⁵ They will come in contact with the cylinder material or impurities present in the mixture and react or adsorb, thus forming new compounds. By understanding the factors that affect the stability, those having an impact can be minimized with appropriate material selection,^{6,7} cylinder treatment,⁸ and quality control of the source material used to prepare the mixtures.⁵ Stable isotope mixtures used for calibration of analytical equipment have an added degree of complexity: the isotopic composition, in addition to the molecular concentration, must remain stable. Either or both of these may change with inappropriate cylinder preparation methods or source material that may contain critical impurities, such as moisture.

Since accurate and reliable calibration mixtures and working gases are important to all stable isotope measurements, experiments were designed to access the effect of moisture on the isotopic stability. A previous work by Gonfiantini et al.⁵ focused on $\delta^{13}\text{C-CO}_2$ ratios and how they changed with the addition of ¹³C-enriched CO₂ into natural-abundance CO₂. In compressed gas cylinders, moisture is ubiquitous and known to

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cause stability issues;^{5,8} therefore, these experiments focused on using added moisture to highlight the ¹⁸O-¹⁶O exchange between the moisture and CO₂. Gaseous carbon dioxide was chosen as a proxy for these evaluations as it is known that the oxygen in CO₂ and the oxygen in water (H₂O) can undergo exchange reactions¹⁰ as illustrated

$$C^{16}O^{18}O + H_2^{16}O \leftrightarrow C^{16}O^{16}O + H_2^{18}O$$

For stable isotope mixtures to be stable (both in composition and isotopic signatures), there must be no residual moisture to participate in exchange reactions.³ This exchange rate increases with both temperature and pressure.¹⁰ Therefore, any residual moisture in the compressed gas cylinder or source gas can participate in this exchange reaction, altering the isotopic composition of both the residual moisture and CO₂. It was therefore decided to use gaseous CO₂ as a model stable isotope compound to understand the sensitivity of this exchange reaction to moisture, which may be contained within the compressed gas cylinder after cylinder preparation or as an impurity in the gaseous CO₂.

Furthermore, the stability with high-purity carbon dioxide was verified with depletion studies to verify that fractionation in appropriately prepared compressed gas cylinders (which eliminates trace moisture) would not occur during usage. The achievement of cylinder nonreactivity and source material purity was validated with a shelf-life study conducted over 1 year.

Additionally, to use a compressed gas cylinder, it is necessary to reduce and regulate the pressure to a safe and suitable inlet pressure for an isotope ratio mass spectrometer (IRMS) or any other instrument used to measure isotope ratios. Experiments were designed to compare δ^{13} C-CO₂ and δ^{18} O-CO₂ values during pressure reduction by both sequential volume expansion (pressure reduction without the use of a pressure regulator) and with the use of a dual-stage pressure regulator.

RESULTS AND DISCUSSION

Exchange of Oxygen in CO₂ and O in Water. Oxygenenriched water was added to a cylinder of research grade CO₂. The cylinder was then heated for 67 h to favor the kinetics of the exchange reaction to achieve a new thermodynamic equilibrium. It was then allowed to sit for 121 days at room temperature. Samples of the CO₂ were obtained both before and after moisture addition and analyzed for δ^{18} O-CO₂ by IRMS. The initial δ^{18} O-CO₂ value was -23.16% vs Vienna Pee Dee Belemnite (VPDB) and the value after 121 days was +109% vs VPDB; thus validating the significant effect that moisture could have in the stability of the δ^{18} O values of CO₂ in compressed gas cylinders.

Pressure Regulation. The ability to regulate pressure from a compressed gas cylinder without fractionation is important to ensure that the highest quality data are obtained. To evaluate this, pressure from a compressed gas cylinder was reduced through sequential expansions to avoid the use of a pressure regulator and compared with the values after pressure reduction using a regulator. A dual-stage, low-dead-volume piston regulator was selected for the comparison over a singlestage regulator as the dual-stage regulator is designed to lower the pressure in two stages and will provide a more consistent delivery pressure.

The experimental setup is illustrated in Figure 1. All measurements were averages of three individual measurements.



Figure 1. Experimental configuration to study the effect of pressure regulation vs a background that was obtained by sequential gas expansion (without the use of a regulator). Stepwise pressure reduction/expansion: Sequential expansion through Segments a, b, and c. The volume of the system is approximately 3024 mL. Effect of pressure reduction: Segments a and d with an in-line pressure regulator. The volume of the aliquoter in Segment d is 5 mL.

Background levels obtained by sequential expansion for $\delta^{13}C$ and δ^{18} O were $(-24.50 \pm 0.05)\%$ and $(-24.99 \pm 0.05)\%$, respectively. Measurements of pressure-reduced samples obtained using a pressure regulator were done in the range of 0.1-0.8 bar. The triplicate measurements are illustrated in Table 1. To assess the effect of pressure reduction, the measurement values at the designated pressures are subtracted from the background. These values are reported as $\Delta^{13}C$ and Δ^{18} O in Table 1. Background levels were obtained by sequentially expanding the gas through a series of successive volume expansions as described in the Materials and Methods section. This facilitated reduction of the pressure to a usable level without the use of a pressure regulator. At the 99% confidence level, the measurement values as a function of pressure are statistically the same as the background indicating that the use of a regulator does not statistically alter the stable isotopic values.

Stability of δ^{13} C–CO₂ and δ^{18} O–CO₂ During Cylinder Depletion. Typically, as the cylinder pressure decreases, species which may be present on the surface of the cylinder, such as moisture, can desorb from the cylinder surface.⁸ Trace moisture is known to desorb from compressed gas cylinder walls below approximately 4 bar;¹¹ therefore, if there is any residual moisture remaining in the compressed gas cylinder, the CO₂–oxygen isotopic ratios will change as the cylinder is depleted, with more pronounced effects below approximately 4 bar.

To validate the absence of moisture in the cylinder after vacuum baking, a 16 L cylinder was filled to approximately 33 bar with gaseous research grade CO₂. The δ^{18} O and δ^{13} C isotopic ratios were measured by IRMS as the cylinder was depleted at a rate of approximately 50 mL/min (Figure 2). The withdrawal took approximately 35 days. The initial δ^{13} C and δ^{18} O values at 33 bar were -39.28 and $-24.13\%_o$, respectively. The final values were $-39.28\%_o$ for δ^{13} C and $-24.14\%_o$ for δ^{18} O. The average values over the entire cylinder depletion were -39.27 and $-24.15\%_o$ for δ^{13} C and δ^{18} O, respectively, with an overall precision of better than $\pm 0.05\%_o$ for δ^{13} C and $\pm 0.07\%_o$ for δ^{18} O. This stability of δ^{18} O over the entire cylinder contents validated the absence of residual moisture in the cylinder or source gas.

Shelf Life. To validate the stability of the CO₂ over time, δ^{13} C and δ^{18} O isotopic values were measured periodically over

Table 1. Background Analysis of δ^{13} C-CO₂ (VPDB) and δ^{18} O-CO₂ (VPDB) Without the Use of a Pressure Regulator (Gas Expansion Only) and of δ^{13} C-CO₂ E(VPDB) and δ^{18} O-CO₂ (VPDB) Obtained with the Use of a Pressure Regulator^{*a*}

		δ^{13} C (‰)	δ^{18} O (‰)	Δ^{13} C (%)	Δ^{18} O (‰)
	background	-24.50 ± 0.05	-24.99 ± 0.05		
	0.14 bar	-24.48 ± 0.03	-25.01 ± 0.06	0.00 ± 0.08	0.02 ± 0.11
	0.21 bar	-24.49 ± 0.04	-25.00 ± 0.07	0.01 ± 0.09	0.01 ± 0.12
	0.34 bar	-24.47 ± 0.04	-24.98 ± 0.06	0.00 ± 0.09	-0.01 ± 0.14
	0.54 bar	-24.47 ± 0.04	-24.98 ± 0.06	0.01 ± 0.09	-0.01 ± 0.11
	0.82 bar	-24.51 ± 0.05	-25.01 ± 0.05	0.01 ± 0.10	0.02 ± 0.10
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 $^{a}\Delta^{13}$ C and Δ^{18} O represent the difference between pressure reduction by sequential volumetric expansion and pressure reduction with a pressure regulator.



Figure 2. δ^{13} C(VPDB) and δ^{18} O(VPDB) vs CO₂ cylinder pressure in bar of a gas-phase CO₂ cylinder, where 35 bar represents a full cylinder. The flow rate of CO₂ throughout the duration of the experiment (~5 weeks) was kept constant at ~50 mL/min.

1 year (Figure 3). Over the period, δ^{13} C and δ^{18} O registered the values of $(-10.85 \pm 0.05)\%$ and $(-12.64 \pm 0.03)\%$,



Figure 3. Stability of gaseous δ^{13} C–CO₂ (VPDB) and δ^{18} O–CO₂ (VPDB) over 1 year.

respectively, validating the stability of the research grade CO_2 with low moisture levels (\leq 3 ppm) in appropriately prepared cylinders. Proper cylinder preparation is discussed in the Materials and Methods section. Note that these isotope measurements were made from a different CO_2 source than those made during the CO_2 depletion experiment, hence the C and O isotopic differences between the two.

CONCLUSIONS

It was demonstrated that with the carbon dioxide source material containing low levels of moisture, proper cylinder preparation, which included achieving vacuum levels $<1.3 \times 10^{-5}$ bar and pressure regulation, the stable isotope composition of both C and O will remain consistent to within ± 0.05 and $\pm 0.07\%$, respectively, throughout depletion of the entire cylinder contents. The belief that gaseous stable isotopic standards are unstable is likely due to mixtures or pure gases with high levels of moisture and poorly prepared cylinders.

Additionally, it was demonstrated that pressure reduction using a dual-stage regulator yielded statistically consistent results at the 99% confidence level from a delivery pressure of 0.1-0.8 bar. It was further shown that the isotopic values remained stable during a 1 year shelf-life study, dispelling longheld beliefs that gaseous components tend to fractionate upon storage and fractionate upon use. This work also illustrates the ability to utilize and regulate pressurized gases as working reference standard gases.

MATERIALS AND METHODS

The cylinders used in this study were 6061 aluminum alloys, which had internal volumes of 47, 30, and 16 L and were obtained from Luxfer (Riverside, CA). All cylinders were visually examined prior to use to ensure that the interior cylinder surface was free from obvious contamination. Ceodeux (Mt. Pleasant, PA) brass diaphragm valves were used. Cylinders were prepared using the Aculife 3 cylinder preparation technology prior to filling, which entails vacuum baking at vacuum levels $< 8 \times 10^{-6}$ bar. Stable isotope ratio measurements were made on a Thermo Fisher Scientific MAT 253+ isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) analyzed in dual inlet mode. Working reference CO₂ materials were calibrated against NBS-18 $(\delta^{13}C_{VPDB} = -5.014\%_0, \delta^{18}O_{VPDB} = -23.2\%_0)$ and NBS-19 $(\delta^{13}C_{\text{VPDB}} = +1.95\%, \delta^{18}O_{\text{VPDB}} = -2.20\%)$ and NIST 8562 $(\delta^{13}C_{VPDB} = -3.72\%, \delta^{18}O_{VPDB} = -8.43\%)$ and NIST 8563 $(\delta^{13}C_{VPDB} = -41.59\%, \delta^{18}O_{VPDB} = -23.61\%)$, and NIST 8564 $(\delta^{13}C_{VPDB} = -10.45\%, \delta^{18}O_{VPDB} = +0.06\%)$. Final ¹³C and ¹⁸O values using the per mil ($\delta\%_0$) deviation of the isotope ratios are expressed relative to Vienna Pee Dee Belemnite (VPDB).

All calibration standards and gases used in this study were obtained from Airgas (Plumsteadville, PA.). Three different sources of research grade gaseous carbon dioxide were used in this study (99.999%, containing ≤ 3 ppm moisture, Airgas, Plumsteadville, PA). Regulators used in this study were Airgas Model 14 dual-stage, low-dead-volume piston regulators (Airgas, Plumsteadville, PA).

Exchange of Oxygen in CO₂ and O in Water. The ability of gaseous CO2 and H2O to undergo an exchange reaction in a compressed gas cylinder was first validated by adding approximately 1 g of ¹⁸O enriched water (98% atom purity, Berry & Associates, Dexter, MI) to a 47 L aluminum compressed gas cylinder using a 2 mL syringe, which had first been purged with dry nitrogen and heated in a lab oven set to 35 °C for 1 h prior to water injection. The injected quantity of moisture was selected to ensure that the dew point was not reached inside the cylinder, thus preventing droplet or cloud formation. The cylinder itself was placed in a cylinder oven set to 80 °C and purged with dry nitrogen several times before evacuating to 8×10^{-6} bar. The cylinder was vacuum-purged several times with the final evacuation performed overnight to 8×10^{-6} bar. The water injection apparatus was evacuated after it was attached to the cylinder prior to quantitative addition of water. After the water injection, the apparatus was purged with dry gaseous research grade CO₂ to ensure that residual water was swept into the cylinder. Approximately 20 barg of gaseous research grade CO2 was added into the cylinder, which was then inverted on a cylinder inverter multiple times to ensure a homogeneous mixture. To favor the thermodynamics and kinetics of the exchange reaction, the cylinder was heated in an oven at 80 °C for 67 h. It was subsequently removed from the oven and allowed to sit at room temperature for 121 days.

Pressure Reduction with Sequential Volumetric Expansion. Prior to studying the dependence of cylinder pressure on the δ value uniformity throughout the compressed gas cylinder contents, it was necessary to validate the ability to reduce the cylinder pressure to that used by the IRMS without potential fractionation caused by pressure regulation. A 57 bar cylinder filled with research grade gaseous CO_2 (moisture ≤ 3 ppm) was used for the comparison. In order to reduce the cylinder pressure to a suitable pressure without the use of a regulator, a system was designed (Figure 1) to facilitate pressure reduction through stepwise gas expansion. All tubing segments were constructed of 1/4 in. electropolished stainless steel tubing, with the exception of the segment between the cylinder valve and valve 9, which was 1/8 in. The tubing segments between the cylinder valve and valve 9 had a volume of 2 mL. The tubing segments between valves 9 and 8 also had an internal volume of approximately 2 mL. The tee's between valves 8, 6, and 7 as well as 3, 1, and 2 had an internal volume of approximately 10 mL. The expansion vessel in "Segment b" was 1 L. The total volume of the sequential volumetric expansion apparatus was approximately 3024 mL. The aliquot tube in "Segment d" was 5 mL.

Volumetric expansion was achieved by first closing the cylinder valve and valve 7 to the regulator. The entire system was then evacuated to 1.3×10^{-6} bar with a vacuum pump prior to expansion. After sufficient vacuum was reached, all valves were closed. The 57 bar CO₂ cylinder valve was opened and the gas was allowed to expand into the 2 mL tubing segment (the small volume between the cylinder valve and valve 9, 1/4 in. SS diaphragm valve, model SS-DSS-4,

Swagelok, Solon, OH). After expanding and allowing the gas to equilibrate for 30 s the cylinder valve was closed and valves 9, 8, and 6 were opened, allowing the gas to expand into the volume within the expansion vessel between valves 6 and 3. The gas was again allowed to equilibrate for 30 s after which valves 6 and 2 were closed and valves 3, 1, and the valve to the 2 L flask were opened. The gas was again allowed to equilibrate for 30 s. The valve to the 2 L flask was then closed as well as valve 1. The 2 L flask (now containing ~0.5 bar of CO_2 gas) was removed from the line and carried to the dual inlet of the IRMS for isotope analysis.

Direct Pressure Regulation. The effect of pressure regulation was obtained by regulating the pressure after the first expansion ("Segment a") with a Model 14 dual-stage, lowdead-volume piston regulator. Valve 6 was closed; the valves in Segment d were opened as well as the value to the CO_2 cylinder and valves 9, 8, and 7. CO₂ was then purged through the regulator and the gas aliquot tube (shown as Segment d) by opening valves 5 and 4 at a rate of 2 L/min for approximately 30 s to achieve a steady state. After purging was complete, valves 4 and 5 were closed (in that order). The pressure was dialed back to zero outlet pressure; valves 7, 8, and 9, as well as the cylinder valve were closed. The gas aliquot tube (Segment d) was removed from the regulator and carried to the dual inlet of the IRMS for isotope analysis. This procedure was repeated as the pressure was regulated at approximately 0.5 bar increments.

Stability of δ^{13} C–CO₂ and δ^{18} O–CO₂ During Cylinder Depletion. If any adsorbed species are present on the surface of the cylinder, they will tend to be desorbed as the cylinder pressure is decreased. The isotopic concentration uniformity as a function of cylinder pressure was evaluated by depleting a gas-phase carbon dioxide cylinder at a rate of approximately 50 mL/min until the cylinder was depleted. The cylinder, which was equipped with a CGA 320 connector, was placed on a cylinder scale to gravimetrically measure the amount of depleted CO₂. A 1/8" stainless steel tubing was used to connect the CGA to the regulator (Figure 4). The gas



Figure 4. Experimental configuration to evaluate the dependence of δ^{13} C-CO₂ (VPDB) and δ^{18} O-CO₂ (VPDB) on cylinder pressure.

distribution system was evacuated to remove any residual contaminants. The cylinder pressure was regulated to approximately 0.2 barg. Gas aliquots were taken periodically by collecting a sample in a gas aliquot tube, "Segment e". The gas aliquot tube was removed from the regulator and carried to the dual inlet of the IRMS for isotope analysis.

Shelf Life. The stability of CO_2 was evaluated by preparing cylinders using the Aculife 3 cylinder treatment process, which included vacuum baking to a vacuum level of $<1.3 \times 10^{-5}$ bar prior to filling the 30 L aluminum cylinders to 35 bar with research grade CO_2 . The cylinders were kept at room temperature and sampled periodically to assess the stability.

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Notes

The authors declare no competing financial interest.

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