Isotope Fractionation of Gaseous-, Supercritical-, and Dual-Phase Carbon Dioxide in Pressurized Cylinders Exposed to Variable Temperatures

Richard A. Socki‡ and Tracey Jacksier*

ABSTRACT: This work examines the stable isotope fractionation of carbon and oxygen in gaseous, supercritical, and liquid carbon dioxide systems at temperatures from −27.1 to +43.5 °C. For pressurized single-, supercritical-, and dual-phase carbon dioxide, both carbon and oxygen isotope fractionations can be measured and are significant when subjected to variations within this temperature range. The δ¹³C and δ¹⁸O values ranged from −41.55 to −41.38 ‰ (VPDB) and −27.74 to −24.9 ‰ (VPDB), respectively, for gas-phase carbon dioxide from 9.3 to 39 °C. A pressure variation of 27.58 barg to 34.48 barg was measured throughout this temperature range. In order to evaluate the effect of supercritical formation and liquefaction on the stable isotope values, cylinders were filled to varying pressures. When stored at cold temperatures, the δ¹³C value as measured in the headspace of the liquid phase varied from −41.23 to −41.13 ‰ (VPDB) and −41.50 to −41.44 ‰ (VPDB) in the supercritical phase. The δ¹⁸O value was between −25.51 and −25.36 ‰ (VPDB) in the liquid phase and between −24.79 and −24.77 ‰ (VPDB) in the supercritical phase. Temperatures in these experiments were selected to mimic outdoor conditions (winter and summer) that stable isotope laboratory practitioners may encounter when storing compressed carbon dioxide cylinders containing stable isotope working reference gases. The carbon and oxygen isotope composition of carbon dioxide gas within these pressurized cylinders return to their precooled isotope values within ~24 h when warmed to laboratory temperatures (~24 °C). A headspace analysis performed immediately after the carbon dioxide cylinder was removed from the cold environment yielded δ¹³C values that were relatively enriched, while δ¹⁸O values were relatively depleted. This is likely an effect of ¹³C and ¹⁸O being preferentially partitioned in the liquid phase within the cylinder. As the cylinder warmed, both liquid and gas equilibrated, and carbon and oxygen homogenized isotopically. As the cylinder was heated into the supercritical phase, a slight opposite isotope effect at higher pressure and temperatures was noted. That is, a slight ¹³C depletion and ¹⁸O enrichment were observed in the gas phase. However, these isotope variations were just slightly outside of the analytical error. Additionally, a separate gas-phase carbon dioxide cylinder was kept at a constant laboratory temperature as a control. This carbon dioxide showed no measurable carbon or oxygen isotope variation throughout the duration of the experimental work. The measured isotope fractionation was significantly higher comparing the phase transition from the gaseous to liquid phase versus the gaseous phase to supercritical phase. The proper handling of pressurized carbon dioxide cylinders used as reference gases for an isotope ratio mass spectrometer includes using carbon dioxide at pressures of less than ~34.88 barg to ensure that the gas is present as a single phase, storing the gas in a temperature-controlled environment, and allowing the gaseous carbon dioxide to equilibrate to ambient conditions for 24–48 h if storage in a controlled ambient environment is not feasible.

INTRODUCTION

With the advent and growth of continuous flow-isotope ratio mass spectrometry (CF-IRMS) techniques over the past few decades,1 there has been an increase in the use of stable isotope analysis in areas such as food authentication and environmental research.2 At the heart of this technology is the requirement for reliable and stable working IRMS reference gas standards. IRMS reference gases are typically contained in high-pressure compressed gas cylinders and connected directly to the IRMS (or an offline aliquot is taken) for use as working gases for direct comparison to unknown samples during stable isotope measurements in either CF-IRMS or dual inlet-IRMS modes. Furthermore, some of these cylinders can contain more than one physical phase at common storage temperatures depending on the pressure and temperature (i.e., gas, supercritical, and liquid), such as carbon dioxide (CO₂) and nitrous oxide (N₂O). However, over the decades, little attention has been paid to the behavior of these multiphase working gases in the compressed state, particularly with respect...
to their potential isotope fractionation at variable temperatures when they are sampled as a gas phase for stable isotope analysis. In order to better understand this behavior, a series of experiments were conducted using single- and dual-phase compressed gas cylinders (30 L) of CO₂. Carbon dioxide within compressed gas cylinders remains an important anchor for isotope ratio measurements of carbon- and oxygen-bearing compounds, and thus, understanding the stable isotope composition of CO₂ reference gas over a range of environmental conditions is paramount to the operation of a scientifically credible stable isotope laboratory.¹

The physical state of any substance is governed by both its pressure and temperature. An understanding of the relationships thus provides the pressure and temperature for phase transitions. For carbon dioxide, the critical point occurs at 30.98 °C and 73.8 barg (barg or bar gauge pressure is the pressure in bars above ambient pressure). Above this temperature and pressure, all of the carbon dioxide will be present in the supercritical phase, that is, an intermediate phase between the gas and liquid states. Above the critical point, a gas cannot be liquefied, no matter the pressure applied.² Like a gas, a supercritical fluid will expand to fill the cylinder; however, it is important to note that the density of the supercritical state is significantly higher than that of the gas phase.³

Grootes et al.⁴ focused on the equilibrium fractionation of carbon and oxygen isotopes in the CO₂(l)↔CO₂(g) system. They showed that at isotopic equilibrium, ¹⁸O tends to be preferentially enriched in the liquid phase (CO₂(l)) and depleted in the gas phase (CO₂(g)), while ¹³C tends to be enriched in the gas phase (CO₂(g)) and depleted in the liquid phase (CO₂(l)). The objectives for this work were to design and execute experiments to determine if carbon- or oxygen-isotope fractionation occurs when sampling CO₂ gas from single-, supercritical-, and dual-phase compressed gas cylinders stored at different temperatures. The range of temperatures was selected to represent conditions that may be experienced as stable isotope laboratory practitioners move stored CO₂ cylinders earmarked for isotope reference use from an outdoor facility to their indoor laboratories. These results provide a basis for recommending proper cylinder handling and storage procedures for CO₂ reference gas for stable isotope laboratory use. Results from these experiments clearly indicate that certain precautions should be taken when dealing with dual-, supercritical-, and single-phase compressed CO₂ cylinders being used as IRMS working laboratory isotope reference gases.

### RESULTS AND DISCUSSION

**CO₂ Cylinder Temperature and Pressure Variations.** All data for samples taken from the three cylinders are shown in Tables 1−3. The CO₂ gas showed systematic and measurable pressure changes when pressurized cylinders were exposed to temperature fluctuations. The temperature vs time graph for all three cylinders is illustrated in Figure 1. Prior to the beginning of the experiment, all three cylinders were stored in the laboratory for ∼4 weeks to ensure thermal equilibrium. The temperature of all three cylinders was 24.3 °C; cylinder A had a pressure of 34.48 barg, and cylinders B and C had a pressure of 57.22 barg. While in the freezer (−26.3 °C), the pressure of cylinder A dropped to 15.86 barg. Once the cylinder was removed from the freezer, the pressure gradually rose to 27.58 barg at 9.3 °C and eventually leveled out to 34.48 barg when warmed to ambient laboratory temperatures. When placed outdoors, the temperature reached 39.9 °C and the pressure remained at ∼34.48 barg.

While in the freezer (−27.1 °C), the pressure in cylinder B dropped to 15.85 barg, like cylinder A and cylinder B following the same pattern with respect to temperature and pressure when removed from the freezer (Figure 1). After 2 h in the warm laboratory ambient environment, cylinder B warmed to −0.6 °C and the pressure rose to 34.48 barg. However, unlike the lower-pressure cylinder A, this higher-pressure cylinder rose to much higher pressures when placed outdoors in the heat of the summer (up to ∼93.00 barg at 43.5 °C). It should be noted that the initial pressure was 57.22 barg for cylinder B and 34.48 barg for cylinder A. Cylinder B experienced a slightly higher outdoor temperature (43.5 °C for cylinder B vs 39.9 °C for cylinder A).

**CO₂ Carbon and Oxygen Isotope Variations.** The CO₂ gas showed systematic and measurable isotope variations when cylinders A and B were exposed to temperature fluctuations. The δ¹³C value of CO₂ in cylinder A (gas only) increased, while the δ¹⁸O value decreased when the cylinder was

---

### Table 1. Summary of CO₂ Cylinder “a” Experimental Data: BG = Background Temperature, Pressure, δ¹³C and δ¹⁸O Composition of Cylinder “A’s” CO₂ before the Beginning of the Experiment

| time (h) | T °C | δ¹³CVPDB ‰ | δ¹⁸OVPDB ‰ | P (barg) | state       |
|----------|------|------------|------------|---------|-------------|
| BG       | 24.3 | −41.54     | −24.77     | 34.48   | gas         |
| 0        | −26.3| −41.34     | −25.07     | 15.86   | gas/liquid  |
| 2        | 9.3  | −41.52     | −24.79     | 27.58   | gas         |
| 24       | 23.0 | −41.54     | −24.90     | 34.48   | gas         |
| 49       | 24.4 | −41.55     | −24.80     | 34.48   | gas         |
| 144      | 23.9 | −41.53     | −24.78     | 34.48   | gas         |
| 480      | 23.7 | −41.51     | −24.80     | 34.48   | gas         |
| 888      | 24.1 | −41.47     | −24.82     | 34.48   | gas         |
| 1344     | 34.5 | −41.41     | −24.77     | 34.48   | gas         |
| 1536     | 39.4 | −41.43     | −24.80     | 34.48   | gas         |
| 1550     | 38.7 | −41.43     | −24.87     | 34.48   | gas         |
| 1574     | 39.9 | −41.44     | −24.89     | 34.48   | gas         |
| 2320     | 39.0 | −41.38     | −24.74     | 34.48   | gas         |

---

### Table 2. Summary of CO₂ Cylinder “B” Experimental Data: BG = Background Temperature, Pressure, δ¹³C and δ¹⁸O Composition of Cylinder “B’s” CO₂ before the Beginning of the Experiment

| time (h) | T °C | δ¹³CVPDB ‰ | δ¹⁸OVPDB ‰ | P (barg) | state       |
|----------|------|------------|------------|---------|-------------|
| BG       | 24.3 | −41.41     | −24.90     | 57.22   | gas         |
| 0        | −27.1| −41.13     | −25.51     | 15.85   | liquid      |
| 2        | −0.6 | −41.23     | −25.36     | 34.48   | liquid      |
| 24       | 24.5 | −41.40     | −24.96     | 57.23   | gas         |
| 45       | 24.8 | −41.43     | −24.90     | 57.22   | gas         |
| 144      | 22.9 | −41.39     | −24.95     | 57.23   | gas         |
| 480      | 23.7 | −41.34     | −24.95     | 57.23   | gas         |
| 888      | 23.8 | −41.29     | −24.96     | 57.23   | gas         |
| 1344     | 34.5 | −41.37     | −24.89     | 57.23   | gas         |
| 1536     | 42.5 | −41.50     | −24.77     | 82.74   | supercritical |
| 1550     | 38.6 | −41.50     | −24.80     | 59.29   | supercritical |
| 1574     | 40.3 | −41.50     | −24.82     | 82.74   | supercritical |
| 2322     | 43.5 | −41.44     | −24.79     | 93.08   | supercritical |

---

ACS Omega 2021, 6, 8625−8629
Table 3. Summary of CO₂ Cylinder “C” Experimental Data: BG = Background Temperature, Pressure, δ¹³C and δ¹⁸O Composition of Cylinder “C”’s CO₂

| time (h) | T °C | δ¹³CVPDB ‰ | δ¹⁸OVPDB ‰ | P (barg) | state |
|---------|------|-------------|-------------|----------|-------|
| BG      | 24.3 | -41.45      | -25.92      | 57.32    | gas   |
| 0       | 23.6 | -41.41      | -24.83      | 57.32    | gas   |
| 2       | 23.8 | -41.40      | -24.96      | 57.32    | gas   |
| 23      | 24.3 | -41.40      | -24.90      | 57.32    | gas   |
| 44      | 22.5 | -41.41      | -24.95      | 57.32    | gas   |
| 143     | 23.4 | -41.39      | -24.93      | 57.32    | gas   |
| 480     | 23.7 | -41.36      | -24.95      | 57.32    | gas   |
| 888     | 23.8 | -41.30      | -24.99      | 57.32    | gas   |
| 1544    | 23.8 | -41.30      | -25.03      | 57.32    | gas   |
| 1538    | 23.6 | -41.34      | -25.02      | 57.32    | gas   |
| 1550    | 23.3 | -41.30      | -25.01      | 57.32    | gas   |
| 1574    | 23.3 | -41.32      | -24.96      | 57.32    | gas   |
| 2324    | 22.4 | -41.31      | -24.96      | 57.32    | gas   |

“This Cylinder Was Subjected to neither Cold nor Heat and Remained within the Ambient Conditions of the Laboratory throughout the Duration of the Experiment.

Figure 1. Temperature vs time for cylinders A, B, and C. Background temperatures were measured prior to the beginning of the experiments. While in the freezer, the pressure of the CO₂ in cylinders A and B dropped to a low of 15.86 barg. The pressure in cylinder A gradually rose to 27.58 barg at 9.3 °C, and eventually leveled out to 34.48 barg when warmed to laboratory temperature and when placed outdoors in the summer heat (up to 39.9 °C). Cylinder B behaved similarly to A; however, due to the higher starting pressure and slightly higher outdoor temperature (43.5 °C), for cylinder B, the pressure rose to 93.08 barg. Cylinder C saw virtually no temperature change from the background throughout the duration of the experiment.

immediately removed from the freezer (Figures 2 and 3). Once the cylinder at 24.3 °C and 34.98 barg (cylinder A) was placed in the freezer at ~26.3 °C, the pressure of the cylinder decreased to 15.86 barg and liquefied, as seen in cylinder A. We interpret these trends in carbon and oxygen isotopes as an effect of 18O and 13C being concentrated within the liquid phase and conversely depleted in the gas phase (initially). Like cylinder A, CO₂ in cylinder B also showed an increase in the δ¹³C value and a decrease in the δ¹⁸O value when first sampled and removed from the freezer. As with cylinder A, initially the 18O and 13C are being concentrated within the liquid phase and conversely depleted in the gas phase. Therefore, when the headspace CO₂ sample was taken from the cold cylinder, it tends to be fractionated (13C enriched and 18O depleted). Essentially the liquid is being sequestered when cold where an isotope fractionation is observed. During this liquid sequestration, the gas, which is being sampled as headspace from the cylinder, tends to be more enriched in 13C and depleted in 18O. These observations are consistent with those seen by Groottes et al. Unlike cylinder A, however, cylinder B, which contained higher pressure, shows a greater degree of isotope fractionation in both 13C and 18O. As cylinders A and B warmed to room temperature over ~24 h, both carbon and oxygen homogenized isotopically and returned to their initial isotopic values. 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, which essentially helps to maintain the homogeneity of the mixture.

After continued periodic measurement of the CO₂ in both cylinders A and B for 846 h (36 days), the carbon and oxygen isotope composition remained constant, within analytical error, that is, ±0.05 ‰ for δ¹³C and ±0.07 ‰ for δ¹⁸O (Figures 2 and 3). After this period, both cylinders A and B were placed...
outside and heated for ∼984 h (~41 days). Temperatures rose to a high of 39.9 °C for cylinder A and 43.5 °C for cylinder B. It should be noted that the difference in temperature between cylinders A and B while outdoors is likely due to each cylinder’s exposure to direct sunlight and cloud cover, while the temperature, pressure, and samples were being taken from each. Furthermore, the temperature inside of the cylinder might not have achieved uniform distribution during this period. Analyses were conducted throughout this period. For heated cylinders (up to 43.5 °C for cylinder B), there may be a slight inverse isotope effect at higher pressure and temperature, as opposed to that seen in low temperature CO2. Both carbon and oxygen isotopes in the CO2 gas in cylinder B tended to show a slight opposite trend as that seen in the cold samples when they were heated outdoors (13C depletion and an 18O enrichment); however, these isotope variations are not significantly outside of the analytical error of the overall measurements.

Cylinder C, which was identical to cylinder B in terms of filling pressure, was used as a control by maintaining it in the laboratory environment through the duration of the experiments. This CO2 cylinder was subjected to neither cold nor heat. The CO2 from cylinder C shows neither 13C enrichment nor 18O depletion (Figures 2 and 3), within analytical error. Isotope trends for both 13C and 18O are also consistent with no liquid isotope fractionation (±0.05 %e for δ13C and ± 0.07 %e for δ18O).

## CONCLUSIONS

The work described herein addresses understanding the behavior of the carbon and oxygen isotopes in pressurized CO2 cylinders when those cylinders are subjected to variations in temperatures that may be encountered during normal earth surface conditions. This understanding is essential since these compressed gases are used as isotope reference materials for routine daily operation in stable isotope laboratories worldwide and since isotope variations can occur due to temperature variations when moving cylinders from, for example, cold outdoor conditions to a warm indoor laboratory. Results from these experiments clearly indicate that certain precautions should be taken when dealing with both dual-phase and single-phase CO2 when used as a laboratory isotope reference gas.

This work shows that for CO2, isotope fractionations can be significant and measurable. The carbon and oxygen isotope composition of CO2 gas within these cylinders quickly returned to their precooled values when warmed to laboratory temperatures. Initial cold cylinder samples, when first removed from a freezer, tended to be the most depleted in 18O and enriched in 13C. We interpret these results as an effect of the 13C and 18O being concentrated within the liquid phase and consequently depleted in the gas phase that was sampled. As the cylinder warmed to laboratory temperature, both liquid and vapor equilibrated isotopically. For cylinders that were heated outdoors up to +43.5 °C, we observed a slight inverse isotope effect at higher pressure, as opposed to that seen in low-temperature CO2.

To eliminate these isotope fractionations, we recommend the following precautions be taken when pressurized CO2 is used as a laboratory isotope reference gas:

- Utilize single-phase CO2 (i.e., less than ~34.88 barg).
- Store CO2 indoors, if possible.

- Always stabilize gaseous CO2 cylinders to laboratory temperature for at least 24—48 h if stored in either cold or hot conditions.

**Experimental Section.** Three separate compressed gas cylinders of CO2 were utilized in this experiment, as outlined in Table 4. All cylinders used in this study were 6061 aluminum alloys and had internal volumes of 30 L (Luxfer, Riverside, CA). Ceodeux (Mount Pleasant, PA) brass diaphragm valves were used. Research-grade carbon dioxide (99.999%) was obtained from Airgas (Plumsteadville, PA). Three CO2 cylinders were utilized to determine the variation of δ13C and δ18O values at different temperatures, pressures, and phases. Initial headspace samples of CO2 gas were taken from all three cylinders prior to the beginning of each experiment and analyzed for δ13C and δ18O. Measurements continued while the cylinders were subjected to variable temperatures.

Two single-phase CO2 cylinders with differing pressures (cylinders A and B) were placed in a top loading freezer and cooled for ∼192 h (~8 days) at −27.1 °C, ensuring that fractionation would occur as the cylinder contents came to thermal equilibrium with the temperature of the freezer. At this temperature, only cylinder B contained liquefied CO2. The two cylinders were subsequently removed from the freezer and brought into the laboratory, and the samples of the gas phase were immediately taken from the headspace of each cylinder. The cylinders were then permitted to warm to laboratory temperature (22.5 °C to 24.3 °C) over an 864 h (36-day) period. These two cylinders were then placed outdoors where temperatures ranged to a maximum of between 39.9 and 43.5 °C, where the headspace was also periodically sampled for another 984 h (41 days). A third gas-phase CO2 cylinder (cylinder C) remained indoors as a control at laboratory temperature throughout the duration of the experiment.

The temperature of the CO2 cylinder was taken using a digital infrared thermometer (Extech Instruments, Model 4250A) at 30.5 cm from the cylinder body until the temperature reading stabilized (~20 s). The precision of the measurements was ±0.8 °C.

Headspace CO2 samples were taken by flowing gas from the cylinder at a low delivery pressure (~0.25−0.34 barg) through a stainless steel gas aliquot assembly (~5 mL in sample volume) consisting of two valves that were connected to the regulator on the cylinder being sampled (Figure 4). After flowing the gas through the aliquot assembly with both valves 1 and 2 open for ~30 s, the valve furthest from the regulator was closed (valve 2). Immediately thereafter, valve 1 was closed and the entire aliquot pressure was adjusted to a zero-outlet pressure. The cylinder valve was subsequently closed, and the entire aliquot assembly was removed from the regulator and connected to the inlet portion of the IRMS by 1/4” compression connection (Swagelok, Solon, Ohio) for isotope analysis. The volume between the 1/4” compression

| cylinder | phase | room temperature pressure | temperature ranges |
|----------|-------|---------------------------|--------------------|
| A        | gas   | 34.48 barg                | −27.1 to 39.9 °C   |
| B        | gas   | 57.22 barg                | −27.1 to 43.5 °C   |
| C        | gas   | 57.22 barg                | 22.5 to 24.3 °C    |
connection and valve 2 on the gas aliquoter was evacuated by the inlet system within the IRMS and then a few millimoles of CO₂ gas was expanded into the dual inlet bellows of the IRMS as matched by the working reference gas.

All gas samples were analyzed via a dual inlet on a Thermo Scientific 253 Plus 10 kV IRMS. Carbon and oxygen isotopes are reported relative to VPDB� with both internal and external standard deviations of measurements typically better than ±0.05 ‰ for δ¹³C and ±0.07 ‰ for δ¹⁸O.

■ AUTHOR INFORMATION

Corresponding Author
Tracey Jacksier — Air Liquide, Innovation Campus Delaware, Newark, Delaware 19702, United States; orcid.org/0000-0001-9930-3172; Phone: +1 302 286 5443; Email: tracey.jacksier@airliquide.com

Author
Richard A. Socki — Air Liquide, Innovation Campus Delaware, Newark, Delaware 19702, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c00474

Author Contributions
‡Analytical Isotopes Inc., Bishop Arts R&D Center, 911 Stewart Drive, Dallas, Texas 75208, United States

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Christopher Romanek for his insightful comments in reviewing an earlier version of this manuscript.

■ REFERENCES

(1) Brenna, J. T.; Corso, T. N.; Tobias, H. J.; Caimi, R. J. High-precision continuous-flow isotope ratio mass spectrometry. Mass Spectrom. Rev. 1997, 16, 227–258.
(2) Moore, J.; Spink, J.; Lipp, M. Development and application of a data base of food ingredient fraud and economically motivated adulteration from 1980-2010. J. Food Sci. 2012, 77, R118–R126.
(3) Werner, R. A.; Roth, M.; Brand, W. A. Extraction of CO₂ from air samples for isotopic analysis and limits to ultra high precision δ¹⁸O determination in CO₂ gas. Rapid Commun. Mass Spectrom. 2001, 15, 2152–2167.
(4) Gas Encyclopedia, Air Liquide, Paris https://encyclopedia.airliquide.com/carbon-dioxide
(5) Wischnewski, R., Wagner, R. Peace Software, einigewerte/calc_CO2.php7
(6) Grootes, P. M.; Mook, W. G.; Vogel, J. C. Isotopic Fractionation Between Gaseous and Condensed Carbon Dioxide. Z. Physik. 1969, 221, 257–273.
(7) Cole, D. R.; Chakraborty, S. Rates and Mechanisms of Isotopic Exchange. Rev. Mineral Geochem. 2001, 43, 83–223.
(8) White, W. A. Stable Isotope Geochemistry in Geochemistry, 1st ed.; Wiley-Blackwell: Chichester, 2013; 361–420.
(9) Socki, R.; Matthew, M.; McHale, J.; Sonobe, J.; Isaji, M.; Jacksier, T. Enhanced Mixture Stability of Stable Isotopic Gases. ACS Omega 2020, 5, 17926–17930.
(10) Coplen, T. B. New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. Geochim. Cosmochim. Acta 1996, 60, 3359–3360.