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Reusable, refillable 12 gm CO2 cylinders as a portable CO2 delivery system for LI-6400 Portable Photosynthesis System (LiCor Biosciences) in regions with limited access to disposable 8 gm/12 gm CO2 cylinders

Important** Working with highly compressed gases can be dangerous, and should not be taken lightly. Compressed gas containers can puncture, and the escaping gas can turn the cylinder into a dangerous projectile. Compressed CO2 is stored in a liquid state, and can cause frostbite if sprayed directly onto exposed skin. Always wear gloves and use eye protection when handling these materials, and do not use or leave compressed air containers in an area with children and animals. They can be injured or killed by a compressed air container that has not been safely stored or is being improperly handled. Considering the inherent danger of working with compressed gas, I recommend that you read this entire protocol before even attempting to replicate the methods, and decide if you are comfortable working with these materials and risks. Always be on the lookout for leaking gas, which can cause dangerous decompression, or even fill a poorly-ventilated room with non-breathable gas. **Important

The LI-6400 Portable Photosynthesis System (Li-Cor Biosciences) is a robust, portable machine for quickly measuring net photosynthesis on living plants, and the accuracy and speed of these measurements is improved when the machine is connected to a source of compressed CO_2. LI-6400 users often rely on disposable metal cylinders (8 or 12 grams variants) containing compressed CO_2 (produced by Li-Cor or various distributors of medical- or food-grade CO2 cylinders) or use a large compressed CO2 cylinder (often >10kg), regulator and flow meter (http://www.licor.com/env/newsline/2011/02/measuring-photorespiration-with-the-li-6400xt-system/) to control the flow of CO2 through the 6400-01 Injector Assembly. While this combination of options makes the LI-6400 a versatile system for working in many regions of the world, there remain some logistical problems:

1. Compressed CO2 cylinders are not permitted on airplanes, due to their highly pressurized contents. Further, these materials are deemed hazardous to ship with most carriers, often requiring permits and considerable customs fees to ship them to your research location. While this is not impossible to overcome, it can consume valuable time and funding.
2. Some developing nations do not have distributors of portable CO2 cylinders, thus making it difficult to supply this important reagent.
3. While many suppliers in developing nations can supply large CO2 canisters for medical, food-preparation and industrial purposes, these canisters can be too heavy and cumbersome to support field measurements in remote, rugged environments.

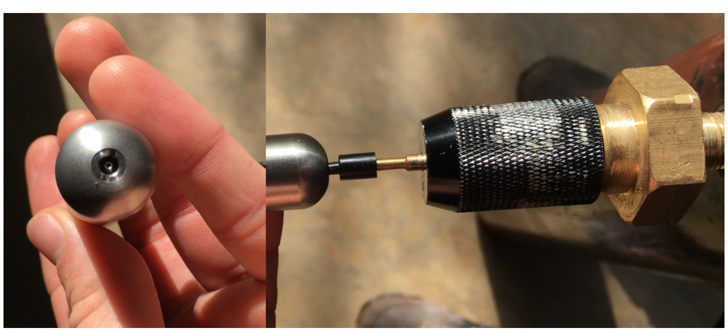
All of these conditions became apparent when I was using the LI-6400 in the Laikipia Plateau of Kenya. Kenya does not host a Li-Cor materials supplier, so I was advised by Li-Cor to seek a supplier of 8 gm food-grade CO2 cylinders to serve as a substitute CO2 source. The only supplier in Nairobi (ISI Group) was unable to supply the cylinders, and couldn't estimate when they would be available. I purchased a 40-kg medical-grade CO2 canister easily in the industrial sector of Nairobi, but the tank was too large to rapidly move through our field sites to take measurements in a landscape-scale natural experiment. I developed the simple assembly detailed below, which circumvented all of the aforementioned problems.

A note on safety: this setup was used repeatedly for 3 months of fieldwork in Kenya, without a single instance of explosive decompression or leaking: while this design lacks a regulator, which increases the inherent risk of using it, the RAP 4 Charger detailed below prevents gas leaks when it is not attached to a refillable cylinder. You may decide for yourself if this design, which was successful in this single pilot project, is suitably tested for your own work. Feel free to contact the author (Patrick Milligan, [patdevmill@gmail.com)](mailto:patdevmill@gmail.com) if you have questions.

Materials:

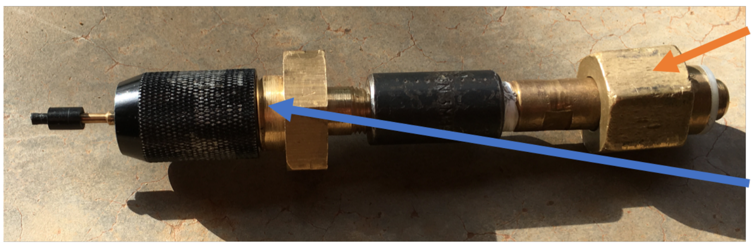
- 12-gm re-usable, re-fillable CO_2 cylinder(s), manufactured by RAP4 Sports http://www.rap4.com/p/017787/rechargeable-12g-co2-cylinder
    - While these are reusable, they can wear out after 20-30 uses. I recommend that you bring a supply of replacements. Further, while these cylinders are rated to safely hold 12 grams of compressed CO_2, I do not recommend filling them to this capacity. If you are planning to take measurements for the entire day, I recommend that you partially fill multiple cylinders (3-4 grams each), and simply switch out the cylinders when the machine starts to show a decline in CO_2 mixer performance.

- 12-gm cylinder Charger, manufactured by RAP4 sports http://www.rap4.com/p/018203/reusable-rechargeable-12g-co2-cylinder-charger?osCsid=oqpg9pc3bj1v9nfrc574k3al00
    - note: this is a proprietary part, produced by RAP4 for use with their proprietary refillable cylinder. The "female" receptacle fits the American standard CGA 320 "male" valve found on CO_2 canisters in the USA. This threaded fitting is NOT compatible with standard CO_2 canister valves in other regions: British Standard BS 341 Part 1 No. 8 (Europe, Asia, and Australasia), DIN 477 No. 6 (A direct European equivalent, interchangeable with BS 341), and Japanese JIS B 8246 (Japan). Read below on how to circumvent this issue using a simple converter.



Refillable cylinder refill port (l) and RAP 4 Charger (r), with its charging muzzle inserted into the port on the cylinder

- A valve converter with two ends: a male CGA 320 threaded outlet and a female BS341 Part 1 No. 8/DIN477 No. 6/JIS B 8246 threaded intake. The intake should be determined based on your region of study (e.g., this setup was developed in Kenya, so we chose a BS341 Part 1 No. 8 female thread). The converter pictured below (admittedly low-tech, consisting of an intake, a connector, and an outlet, sealed with PTFE thread tape) cost about USD 10.00 in the Nairobi industrial sector. This should be built by a machinist experienced with compressed gas systems. Make sure that the converter can handle pressures of at least 2000 psi, as many large CO2 canisters are filled to around 1200 psi.



"Female" BS 341 Part 1 No. 8 threaded intake

"Male" CGA 320 threaded outlet, connected to a RAP 4 Refillable 12 gram cylinder "Charger"

- Silicone lubricant to maintain O-rings in the CO2 cylinder charging port

- Adjustable-width wrench

- PTFE thread-sealing tape

- Precision needle-nose pliers set, used to adjust the valve on the RAP 4 Charger (e.g., https://www.amazon.com/6-Piece-Precision-Pliers-Set/dp/B0015YKBAE)

- Digital scale

Methods: (note: this protocol can take >6 hours to complete, so it would be prudent to do this at least the day *before* field measurements with the LI-6400 are scheduled to begin)

1. Assembly of converter and cylinder charger

    a. Turn the female intake of the RAP 4 Charger towards you, and you will be able to see a 2-cm wide brass circular plate on the inner wall of the intake, with two small circular grooves drilled into the disc. Insert each pointed nose of the precision needle-nose pliers into these grooves, and rotate the plate one-quarter turn counter-clockwise. This allows a small amount of pressurized CO2 to be channeled through the charger. This will **only** allow CO2 to escape when a refillable CO2 cylinder is connected to the Charger port at the end of this protocol.

    b. Wrap the male CGA 320 thread outlet of the valve converter 3 times with PTFE thread-sealing tape, and press down on the tape to ensure that it fills the gaps in the valve threads. This helps to prevent minor leakage of compressed CO2 through the valve threads.

c. Screw the female intake of the RAP 4 Charger onto the male CGA-320 outlet of the valve converter. The thread tape will be further pressed down into the threads by this action. Tighten the Charger onto the converter using the adjustable wrench. Considering the highly pressurized CO2 contents, tighter is always better when attaching the Charger.

d. Wrap the CO2 tank outlet valve threads with PTFE thread-sealing tape 3 times, and press the tape down into the threads to ensure that there is uniform coverage.

e. Attach the BS 341/DIN 477/JIS B 8246 female intake of the valve converter to the CO2 tank outlet, and tighten with the adjustable wrench. Again: considering the highly pressurized CO2 contents, tighter is always better when attaching the valve converter to the CO2 tank.

CO2 canister (l) and BS 341 Part 1 No. 8 threaded outlet (r)

2. Prepare refillable CO2 cylinders for filling

   a. RAP 4 refillable CO2 cylinders can be dissembled for cleaning and storage: simply grip both ends of the cylinder and unscrew counter-clockwise. (note: this is probably the ideal way to carry these refillable cylinders in checked luggage on an airplane, or to ship them via mail carriers, so that they will not be mistaken for pre-filled disposable cylinders. Take care that any refillable cylinders that you travel with or ship have been completely discharged and dissembled.)

b. Place the dissembled cylinder pieces in a sealed Ziploc bag or plastic container, and then place them in a freezer or cooler filled with ice for at least 4 hours. Compressed CO_2 is released at temperatures well below 0°C, and if a CO_2 cylinder is filled at room temperature it may cause damage to the O-ring and explosive decompression. Cooling the cylinder allows the metal cylinder and rubber O-ring to gradually reach a lower temperatures and prevent damage.

c. Remove the cylinder pieces from cold storage, and carefully screw them together. Take care to correctly align the threads on both sides of the cylinder, so that gas will be safely sealed within the cylinders after filling.

Partially re-assembled cylinder. Make sure that the cylinder is fully assembled before trying to fill it (i.e., the black rubber O-ring should **not** be visible during use)

3. Fill RAP 4 refillable CO_2 cylinders

a. Place a small dot of silicone grease onto the filling port on the refillable CO_2 cylinder. Use the silicone grease applicator tube to coat the hollow rod in the filling port with grease. This further protects the rubber O-ring from damage during filling.

b. Record the weight of each refillable CO_2 cylinder before proceeding to the following steps. The weight of these cylinders can vary slightly around ~50 grams. I.e., any gains in weight after filling these cylinders can be recorded as the current amount of CO_2 contained in that cylinder.

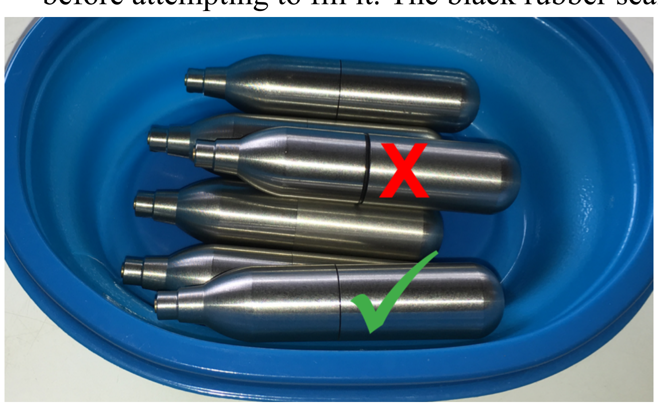
c. Slowly open the release valve on the CO_2 tank by turning the handle counter-clockwise, one-quarter turn at a time, until the valve handle has been turned one full revolution. This allows CO_2 to fill the converter assembly.

   i. *If at any point you hear gas escaping from the valve converter or the RAP 4 Charger, immediately turn the CO_2 release valve handle clockwise and prevent any further gas escape. This may indicate a leak, and the entire setup may need to be reassembled.*

d. Gently place the refill port of the refillable CO2 cylinder against the charging muzzle on the RAP 4 Charger, so that the muzzle completely encompasses the hollow pin in the cylinder refill port. Apply pressure on the CO2 cylinder, pushing it towards the Charger. This will form a seal between the Charger muzzle and the cylinder O-ring, and you will feel the rod on the Charger depress into the female housing: this allows CO2 to flow from the cylinder through the Charger into the refillable CO2 cylinder. Maintain pressure for 5 seconds, and then release pressure. This will close the valve on the Charger and prevent any additional gas from escaping.

i. Weigh the cylinder again, and subtract the initial weight to determine the amount of CO2 in the cylinder. I recommend that you only fill each cylinder with 3-4 grams of compressed CO2. Again, these cylinders are rated to hold 12 grams of compressed CO2, but I simply recommend that you do not approach this upper limit in case your cylinders are heated or compressed during fieldwork.

ii. Remember to fully screw together the two parts of the portable cylinder before attempting to fill it. The black rubber seal should not be visible.



4. Turn off the CO2 release valve

    a. Turn the CO2 canister release valve clockwise, until the handle can't be closed any further. Ensure that this valve is closed as tightly as possible.

    b. Repeat protocol 3c, which will allow the residual of CO2 to escape from the converter assembly and Charger. This will produce a small hiss for 1-2 seconds. If this decompression continues, then remove the refillable cylinder from the Charger muzzle and repeat step 4a, tightly closing the CO2 canister release valve. Then, repeat step 4b, until no more gas escapes from the converter.

    c. If you are planning to store your CO2 canister a long period of time, I strongly advise against storing it with this assembly attached. Use your adjustable wrench to loosen the nut on the CO2 canister valve, and then screw the nut clockwise to remove it from the valve.

    d. Screw the safety hood onto the top of the CO2 canister for long-term storage.

5. Using refillable CO2 cylinders in a Li-Cor 6400-01 CO2 mixer

    a. These refillable CO2 cylinders work just like a disposable CO2 cylinder in the 6400-01. The pin on the 6400-01 usually pierces the thin metal casing at the disposable cylinder tip: instead, the pin will depress the spring-loaded mechanism in the refillable CO2 cylinders, which will form a seal with the 6400-01 O-ring. Follow the steps detailed in the Li-6400 user manual, and take care to change out the air filter in the 6400-01 every 20-25 uses, since residual silicone grease may accumulate in the filter.