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# Dissolved inorganic carbon concentration and $^{13}\text{C}/^{12}\text{C}$ V.1

 In 1 collection

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**Protocol status:** Working

We have used this procedure in our group and it works. However, we have made some revisions reflected in newer versions of the procedure.

**Created:** March 19, 2019

**Last Modified:** May 17, 2019

**Protocol Integer ID:** 21580

**Keywords:** dissolved, inorganic, carbon, isotope, GasBench, delta

## Abstract

CO<sub>2</sub> conversion by H<sub>3</sub>PO<sub>4</sub>, extracted CO<sub>2</sub> introduced into continuous flow IRMS for <sup>13</sup>C/<sup>12</sup>C analysis

This method follows the general principles described in Assayag et al. (2006), which emphasizes the importance of maintaining the same water : H<sub>3</sub>PO<sub>4</sub> volume ratio for standard solutions and samples (ratio of 10 in this case) in order to obviate issues regarding isotopic fractionation between CO<sub>2</sub> (g) and CO<sub>2</sub> (aq).

## References

Assayag, N., Rivé, K., Ader, M., Jézéquel, D., and Agrinier, P. (2006). Improved method for isotopic and quantitative analysis of dissolved inorganic carbon in natural water samples. *Rapid Communications in Mass Spectrometry* 20, 2243–2251


## Guidelines

### Avoiding microbial alteration after sampling

- There are multiple options for ensuring biological activity does not affect DIC measurement after sampling. One surefire approach is poisoning with mercuric chloride (i.e. adding to exetainer as solution and drying it down in the lab before bringing to field), but this can be logistically difficult and potentially hazardous. An alternative is using a 0.2 µm polycarbonate inline syringe filter. Some studies caution against using a filter due to risk of re-equilibration or degassing of sample during filtering. DBN has tested this, and any effects this causes appear to be minimal. Anyway, if you want to be sure that you are measuring dissolved inorganic carbon as opposed to particulates, you need to filter. A 0.2 µm filter may not catch very small microbes, but it is a standard microbiological technique for sterilization, and it should eliminate the vast majority of active microbes.
- Another precaution you can take to limit microbial activity is to add H<sub>3</sub>PO<sub>4</sub> to the sampling vials before taking them to the field. This should bring the pH of the solution <1, where very few microbes can function.
- Some studies like to add the H<sub>3</sub>PO<sub>4</sub> to all water samples at the same time in the lab after sampling to ensure all samples have the same time to equilibrate, but DBN does not think this is necessary since as long as all samples have had sufficient time to reach equilibrium, the total amount of time between acidification and analysis should not matter.
- DBN prefers to pre-acidify and filter samples, since this limits microbial activity without generating toxic waste.
- For 2018 Oman samples, samples were acidified in the lab after sampling; for 2019 Oman samples, 85% H<sub>3</sub>PO<sub>4</sub> was added to Exetainers prior to sampling.

## Materials

### STEP MATERIALS

 Ultra Pure Carrier 5.5 Grade Helium, Size 300 Cylinder, CGA-580 **Airgas Catalog #HE UPC300**

 o-Phosphoric Acid 85% **Fisher Scientific Catalog #A242-212**

 Ultra Pure Carrier 5.5 Grade Helium, Size 300 Cylinder, CGA-580 **Airgas Catalog #HE UPC300**

 o-Phosphoric Acid 85% **Fisher Scientific Catalog #A242-212**

In addition to materials listed in steps, here are some standard lab items you'll need

(sizes depends on number and size of samples)

- Erlenmeyer flask
- beaker
- kimwipes / paper towels
- ring stand
- glass funnel
- tube rack

### Protocol materials

 Ultra Pure Carrier 5.5 Grade Helium, Size 300 Cylinder, CGA-580 **Airgas Catalog #HE UPC300**


 o-Phosphoric Acid 85% **Fisher Scientific Catalog #A242-212**

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 Ultra Pure Carrier 5.5 Grade Helium, Size 300 Cylinder, CGA-580 **Airgas Catalog #HE UPC300**

 o-Phosphoric Acid 85% **Fisher Scientific Catalog #A242-212**

## Plan samples

Before prepping vials, you must consider what range of dissolved inorganic carbon (DIC) you expect in your samples.

The sensitivity range is determined by the method blank, the water volume, the lower limit of mass for which we can consistently measure carbonate standards ( $\sim 10 \mu\text{g}$ ), and the detector saturation / linearity effects on the isotope ratio mass spectrometer (IRMS). DBN has optimized procedures to lower blanks, which are reflected in this procedure, although further blank reduction is an ongoing project.

Below are some guidelines of appropriate volumes to use:

- A 1 mL water sample in a 12 mL Exetainer will be good for most freshwater and marine systems (sensitivity range  $\sim 300 - 4000 \mu\text{M}$  on Thermo GasBench - Delta V IRMS).
- If you expect  $[\text{DIC}] > 4000 \mu\text{M}$ , you can use less sample (i.e. 0.5 mL water).
- If you expect  $[\text{DIC}] < 300 \mu\text{M}$ , you can use a larger sample size, i.e. 100 mL blue-butyl stoppered serum vial with a 50 mL water sample. Note, this requires manual sampling with the GasBench needle.
- If your system has a wide range of  $[\text{DIC}]$ , you can take replicate samples at different water volumes (i.e. for Oman ophiolite, where  $[\text{DIC}]$  ranges  $\sim 3500 - <10 \mu\text{M}$ , DBN takes 1 mL and 50 mL samples for DIC)

## Plan method blanks

Also, consider what you will use as a method blank. At minimum, you must purge (1-3) vial(s) with helium during sample preparation, and subsequently add decarbonated water +  $\text{H}_3\text{PO}_4$ .

If you plan to have a quick turnaround time between preparing samples and analyzing them ( $<1$  week), and are confident that you will return with samples, you can save time by preparing your full standard suite at the same time as you prepare your samples.

If the period between sample preparation and analysis is longer (weeks to months), or if you have any doubt that you will return with samples (due to mishaps in field or new experimental design etc.), you should probably wait to prepare standards until a day before analysis to ensure that your standards are unaltered and to avoid wasting time and standards for a run that never occurs.



If the latter applies to you, then prepare a set of blanks as you prepare your samples and another set of blanks the day prior to analysis to check for 1) diffusion between sample preparation and analysis and 2) the repeatability of your blank preparation. Ideally, blanks should be brought into the field because diffusion is a function of pressure gradients and temperature, which may be different in the lab compared to your sampling site. In addition to the He purge + water blank mentioned previously, He-only, He + H<sub>3</sub>PO<sub>4</sub> only, and/or He + carbonate mineral standard + water + H<sub>3</sub>PO<sub>4</sub> can be prepared to test for CO<sub>2</sub> loss/gain, and to determine the sources error, if necessary.

***The steps below describe the full laboratory procedure for preparing samples and/or standards. Depending on your specific sampling and blank preparation strategy, some steps may not apply. In any case, stop when you reach the centrifugation step, and finish centrifugation and equilibration the day prior to analysis on the IRMS.***

#### Note

For 2018 Oman samples, DBN left blanks in the lab; in 2019, DBN brought blanks to the field.

DBN has not noticed significant change in blanks over ~1 month time scales.

#### Equipment

Exetainer® 12ml Vial - Round Bottom	NAME
vial	TYPE
Labco	BRAND
938W x 1000	SKU
<a href="https://www.labco.co.uk/products/standard-exetainer-cap/product/557-exetainer-12ml-round-bottom/category_pathway-15">https://www.labco.co.uk/products/standard-exetainer-cap/product/557-exetainer-12ml-round-bottom/category_pathway-15</a>	LINK



### Equipment

Wheaton™ Serum Bottles and Vials

NAME

vial

TYPE

DWK Life Sciences

BRAND

06-406J

SKU

<https://www.fishersci.com/shop/products/wheaton-serum-bottles-vials-15/06406j#?keyword=06-406J>

LINK

100 mL nominal volume

SPECIFICATIONS

### STEP CASE

If using 12 mL Exetainer 29 steps

- 2 **Combust with aluminum foil covering the mouth of the Exetainer in muffle furnace 450 °C for 5 hr.**

#### Note

- DBN has found that Exetainers (with septum installed) are consistently  $11.70 \pm 0.01$  (1s) mL. This is sufficiently consistent that the volume of Exetainers do not need to be individually measured before sampling for DIC concentration.
- Since the Exetainers are continually exposed to acid, it is not necessary to give them a separate acid wash.

- 3 **Weigh out carbonate standards on micro balance, and label standard vials.**



## Equipment

XP6 Excellence Plus XP Micro Balance, 6.1g x 1Ug	NAME
micro balance	TYPE
Mettler Toledo	BRAND
BZ10134871	SKU
<a href="https://www.coleparmer.com/i/mettler-toledo-xp6-excellence-plus-xp-micro-balance-6-1g-x-1ug/1133601">https://www.coleparmer.com/i/mettler-toledo-xp6-excellence-plus-xp-micro-balance-6-1g-x-1ug/1133601</a>	LINK


## 4 Cap vials

- Gently screw the cap onto the vial until slight resistance is felt with the septum.
- Then turn the cap an additional one quarter turn.
- Looking at the top of the cap on the sealed vial from above, a very slight indentation of the septum may be seen through the aperture of the cap, indicating a good seal
- If the cap has a puckered look, it has been overtightened. If this is the case, replace with new cap.
- <http://www.labco.co.uk/information/user-guide>

## 5 Purge vials with Helium

He purge the vials for 5 minutes

- Put the purging needles in the septa such that the inflow needle is a bit deeper than the outflow needle. This allows you to pull both needles out at the same time with the outflow needle being extracted first. It is best to avoid wildly different partial pressures of He between vials, so it is good to do this consistently.
- It is good to have needle outflow connected to tube that is submerged in water. this prevents air leakage when removing needles.
- Approximately 5 bubbles per second should emanate from the outflow tubes submerged in water.

 00:05:00



Ultra Pure Carrier 5.5 Grade Helium, Size 300 Cylinder, CGA-580 **Airgas Catalog** #HE UPC300



## Equipment

BD General Use and PrecisionGlide Hypodermic Needles - 23g (0.064 cm x 2.5 cm)	NAME
needle	TYPE
BD	BRAND
14-826A	SKU
<a href="https://www.fishersci.com/shop/products/bd-general-use-precisionglide-hypodermic-needles-20/14826a">https://www.fishersci.com/shop/products/bd-general-use-precisionglide-hypodermic-needles-20/14826a</a>	LINK

## 6 Boil water

- Use acid washed glassware if possible, but ultra-pure (MilliQ) water rinsed is OK if you don't have acid-washed glassware prepped.
- Rinse an Erlenmeyer flask for the  $\text{H}_3\text{PO}_4$  and a beaker for the water with ultra-pure  $\text{H}_2\text{O}$  from Kopf lab, BESC 335.

### Note

Make sure you select an Erlenmeyer flask that is an appropriate size such that the syringe you are using for  $\text{H}_3\text{PO}_4$  transfer can fit in and reach near the bottom of the flask. Otherwise, you will end up wasting  $\text{H}_3\text{PO}_4$ . You may need to prepare multiple batches of  $\text{H}_3\text{PO}_4$  depending on the number of samples you have.

- Rinse 2 small stir bars (<1 cm) in ultra-pure water, then add them to the flask and beaker. Usually the flask will need an even smaller stir bar than the water beaker.
- Cover beaker/flask with aluminum foil but leave a little room for venting. This helps it heat up faster and avoids contamination.

## Safety information

Ultra-pure water can overheat and then violently boil since it lacks impurities which are good nucleation points for boiling. DBN has not had any issues with this happening, but would suggest using older glassware and stirbars that are more likely to have tiny nicks and scratches, which can act as nucleation points for boiling. Alternatively (and probably optimally, although DBN has not used yet for this method), consider adding boiling stones to water and/or  $H_3PO_4$ .

- Put the water on the small VWR hotplate in the hood in rm. 345, heat setting 10, stir setting 1. Depending on volume of water, this may take ~15 min to get boiling. Wait 10 min from first observation of bubbles. Then turn off heat, wait for liquid to stop boiling, and then remove from hot plate.

⌚ 00:10:00

## Equipment

Barnstead™ GenPure™	NAME
Water purification	TYPE
Thermo Scientific	BRAND
50131211	SKU
<a href="https://www.thermofisher.com/order/catalog/product/50131211">https://www.thermofisher.com/order/catalog/product/50131211</a>	LINK



### Equipment

VWR 220 Mini Hotplate Stirrer	NAME
hotplate	TYPE
VWR	BRAND
SKU unknown	SKU

## 7 Inject water in standard vials

- While water is still quite hot, but removed from the hotplate (for 1-3 min), take aliquot with syringe (a luer lock tip, 3 mL syringe is good for 1 mL water injection b/c it makes it easier to purge out bubbles, especially if you are using a syringe filter), and inject into the vial right-side up (cap on top).

### Note

Boiling water helps reduce CO<sub>2</sub> background, but if water or acid is too hot, it will deform the syringe tip, which can result in your needle getting stuck in the vial, which is no good.

### Note

Use 'nurse technique' to remove bubbles from syringe (invert syringe and flick while pushing out liquid. waste container can be useful for this.)

#### Note

If you are using a syringe filter for field samples (strongly recommended), I would recommend also using one in water injections of standards. If doing this, hold on to the needle as you pull it out of the Exetainer to avoid the needle falling off and degassing occurring.

#### Note

If you have a lot of samples, and water injections are taking long enough that your water is cooling down, you may consider putting it back on the hotplate at low heat (~heat setting 2) to keep warm.

#### Note

For water and acid additions in Exetainers, it is OK to re-use needles between vials.

#### Equipment

BD™ Disposable Syringes with Luer-Lok™ Tips - 3 mL	NAME
syringe	TYPE
BD	BRAND
14-823-435	SKU
<a href="https://www.fishersci.com/shop/products/bd-disposable-syringes-luer-lok-tips-3/14823435">https://www.fishersci.com/shop/products/bd-disposable-syringes-luer-lok-tips-3/14823435</a>	LINK



## Equipment

<b>BD General Use and PrecisionGlide Hypodermic Needles - 23g (0.064 cm x 2.5 cm)</b>	NAME
needle	TYPE
BD	BRAND
14-826A	SKU
<a href="https://www.fishersci.com/shop/products/bd-general-use-precisionglide-hypodermic-needles-20/14826a">https://www.fishersci.com/shop/products/bd-general-use-precisionglide-hypodermic-needles-20/14826a</a>	LINK

## Equipment

<b>Basix™ Syringe Filters, PES, Sterile</b>	NAME
syringe filter	TYPE
Fisher Scientific	BRAND
13-100-106	SKU
<a href="https://www.fishersci.com/shop/products/fisher-scientific-basix-syringe-filters-pes-sterile/13100106#?keyword=13-100-106">https://www.fishersci.com/shop/products/fisher-scientific-basix-syringe-filters-pes-sterile/13100106#?keyword=13-100-106</a>	LINK





## 8 Prepare $\text{H}_3\text{PO}_4$

### Safety information

Use safety goggles, lab coat, and nitrile gloves while handling  $\text{H}_3\text{PO}_4$ .

- Using stand with ring attachment and glass funnel (preferably acid-washed) in the hood, carefully transfer 85%  $\text{H}_3\text{PO}_4$  to the Erlenmeyer flask. Be careful not to fill it up all the way (<80% of flask volume) because the  $\text{H}_3\text{PO}_4$  expands and bubbles upon heating.
- Boil according to the same steps as for water.
- Transfer to vials in the same manner as for water, but **do not** use a syringe filter.

### Note

Use water: $\text{H}_3\text{PO}_4$  ratio of 10, i.e. if using 1 mL water sample, use 100  $\mu\text{L}$  85%  $\text{H}_3\text{PO}_4$ .

DBN has confirmed that this reduces pH of hyperalkaline Oman water from >11 to <2, so it should work for most any water.

### Note

As it turns out, hot acid is not the best thing for plastic syringes. If you notice that the luer-lock threads are not connecting well, start using a new syringe to avoid a needle coming loose in the vial.



o-Phosphoric Acid 85% **Fisher Scientific Catalog #A242-212**



## Equipment

BD Disposable Syringes with Luer-Lok™ Tips (1 mL)

NAME

Syringe

TYPE

BD

BRAND

14-823-30

SKU

<https://www.fishersci.com/shop/products/bd-disposable-syringes-luer-lok-tips-3/1482330>

LINK





Adding 85%  $\text{H}_3\text{PO}_4$  to erlenmeyer flask with funnel.





Heating 85% H<sub>3</sub>PO<sub>4</sub>





Injecting 95% H<sub>3</sub>PO<sub>4</sub> into Exetainer.

## 9 Clean off vials

After injections are finished, carefully wipe the tops of the Exetainer with paper towel / kimwipe so that no fluid remains on the septa.

## 10 Centrifugation

- I recommend centrifugation, which serves two purposes: (1) getting liquid off the top of the septum, which can potentially enter the GasBench needle and get into the capillary and (2) ensuring that no  $\text{CaCO}_3$  powder sticks to the sides of the Exetainer, which can throw off your calibration.
- Before centrifuging standards, visually inspect if there are any bits of carbonate stuck to the sides of the exetainer. If there are, try to swoosh the liquid around to scoop it up.
- Centrifuge all samples 5 minutes at 2500 rpm on Templeton lab centrifuge.
- After centrifuging, visually inspect the standard solutions again for sticking particles. If necessary, repeat swooshing liquid and centrifuging until there are no more sticking particles.

### Note

Mass must be balanced on either side of centrifuge. Add another vial to balance centrifuge if necessary.

### Equipment

Eppendorf™ 5810R Centrifuge

NAME

Centrifuge

TYPE

Eppendorf

BRAND

02-262-8187

SKU

<https://www.fishersci.com/shop/products/eppendorf-5810r-centrifuge-rotor-packages-16/022628187>

LINK





12 mL round-bottom Exetainer in centrifuge. Works fine in 15 mL conical tube slots.



## 11 Equilibration

After centrifuging, place exetainers in tube rack on shaker table (upright, with caps on top). Shake at ~150 RPM for 15-18hr. Ideally, do this in the stable isotope lab so it is at same temperature as it will be analyzed at.

### Equipment

**C1 Platform Shaker**

NAME

Platform Shaker

TYPE

New Brunswick Scientific

BRAND

SKU unknown

SKU



Exetainers on tube rack screwed into platform shaker. Speed 40 is good for this model.

- 12 **Ensure that heat block on GasBench is turned off well before samples are analyzed (a few hours to overnight).**

## 13 Centrifuge again

This may sound excessive, but I would recommend centrifuging again right before analysis. The reason is that condensation often forms while the samples are shaken overnight. This especially true for standards that had hot water added to them the previous day. Centrifuging again probably saves time overall by reducing the likelihood of GasBench needle clogging.

## Field sampling

14 **Take to the field He-purged Exetainers** as described in Section 'Laboratory preparation before sampling.'

## 15 Taking samples

- Fill a 3 mL syringe with sample water and attach 0.2 µm polycarbonate in line filter and needle.
- Discard water.
- Refill the syringe with sample water. Ensure there are no bubbles. Attach filter and needle. Discard water until only the sample volume is left in syringe.
- Inject water into exetainer in same manner as standards (right-side up).
- Withdraw syringe swiftly, holding by the needle so it doesn't come loose in the septum.

### Note

If water is being pumped from subsurface and there is concern about the sample equilibrating with atmosphere, then it is probably best to sample directly from the pump stream (i.e. through a luer-lock port on a manifold), but if ease of sampling is an issue and/or the water being sampled is naturally open to air (i.e. sampling a river or sampling from a boat), sampling is easier and more reproducible if a sub-sample reservoir is taken (i.e. a 10L bucket, washed out three times with site water.)

### Note

Consider taking samples in duplicate or triplicate if possible.

**Note**

Be sure to keep needles and filters sterile between sample replicates.

**Note**

Some people like to store samples upside down which allegedly decreases gas leakage across the septa, but I am not aware that anyone has tested this. I would say it does not matter as long as you are consistent with sample storage in all samples. I would recommend bringing a tube rack to field to stabilize samples during transport. If you do store samples upside down, make sure to do the centrifugation step during sample prep.

For Oman samples, DBN stored samples rightside in 2018 and upside-down in 2019.

## Running samples on GasBench - Delta V IRMS

- 16 **Check He tank pressure and write down in Delta notebook**
- 17 **Go to Isodat acquisition program.**
- 18 **Check in bottom left that it is on ConFlo IV + gasbench & CO<sub>2</sub> mode**
- 19 **Turn on reference gas dilutions to make a Voltage in range of what you expect for your samples**
  - ConFlo IV Diagnosis > Reference Dilution
  - Engage reference dilutions 1 and 2
  - Write down the dilution setting in Delta lab notebook



### Equipment

DELTA V™ Plus Isotope Ratio Mass Spectrometer	NAME
Isotope Ratio Mass Spectrometer	TYPE
Thermo Scientific	BRAND
IQLAAEGAATFABHMZZZ	SKU
<a href="https://www.thermofisher.com/order/catalog/product/IQLAAEGAATFABHMZZZ?SID=srch-srp-IQLAAEGAATFABHMZZZ">https://www.thermofisher.com/order/catalog/product/IQLAAEGAATFABHMZZZ?</a> <sup>LINK</sup> SID=srch-srp-IQLAAEGAATFABHMZZZ	

## 20 Turn on Reference gas

- ConFlo IV Interface > turn on ref II status (CO<sub>2</sub>)

### Note

It's a good idea to turn on reference gas right away b/c it helps flush any water vapor out of the system

## 21 Write down HV, Vac, Box, Trap

## 22 Run autofocus

- Icon with 2 blue bars in upper left
- Write down mass 44 voltage in delta lab book

## 23 Pass to gas configuration

## 24 Write "pass to gas config" next to mass 44 voltage in delta lab book

## 25 Turn off reference CO<sub>2</sub> tank

- Give mass spec ~1 min to let this gas flush out.
- Write down the background voltages for Mass 44, 45, and 46

**26 Switch to ConFlo IV mode****27 Run On/Offs & linearity**

- Open → Thermo → Isodat NT → Global → User → ConFlo IV Interface → ConFlo IV Interface → Sequence → 20171024 CO2 On Off Lin.seq
- Run 'CO2 On Off.met' and 'CO2 Linearity.met'
- Say OK to all the run settings
- The point of this is basically to determine if the mass spec is running properly
- For the On/Offs,  $\delta^{18}\text{O}$  should have a standard deviation of ~0.01 per mil
- Linearity results with standard deviation < 0.15 per mil for  $\delta^{18}\text{O}$  is fine

**28 Load Exetainers in GasBench autosampler****Equipment****GasBench II**

Gas delivery system

Thermo Scientific

IQLAAEGAATFAETMAGD

[https://www.thermofisher.com/order/catalog/product/IQLAAEGAATFAETMAGD?gclid=E](https://www.thermofisher.com/order/catalog/product/IQLAAEGAATFAETMAGD?gclid=E%20tBh1YswK_EAAYASAAEgLOjFD_BwE&ce=E.19CMD.DL105.16198.01&cid=E.19CMD.DL105.1%20tBh1YswK_EAAYASAAEgLOjFD_BwE:G:s&s_kw)  
[tBh1YswK\\_EAAYASAAEgLOjFD\\_BwE&ce=E.19CMD.DL105.16198.01&cid=E.19CMD.DL105.1](https://www.thermofisher.com/order/catalog/product/IQLAAEGAATFAETMAGD?gclid=E%20tBh1YswK_EAAYASAAEgLOjFD_BwE&ce=E.19CMD.DL105.16198.01&cid=E.19CMD.DL105.1%20tBh1YswK_EAAYASAAEgLOjFD_BwE:G:s&s_kw)  
[tBh1YswK\\_EAAYASAAEgLOjFD\\_BwE:G:s&s\\_kw](https://www.thermofisher.com/order/catalog/product/IQLAAEGAATFAETMAGD?gclid=E%20tBh1YswK_EAAYASAAEgLOjFD_BwE&ce=E.19CMD.DL105.16198.01&cid=E.19CMD.DL105.1%20tBh1YswK_EAAYASAAEgLOjFD_BwE:G:s&s_kw)

**29 Set up sequence****30 Select all fields in your sequence and start data acquisition.**