

UCI KCCAMS Facility

Seawater Dissolved Inorganic Carbon Protocol

April 07, 2005

Contents

I. DIC Collection Procedure	page 5
II. DIC Extraction Procedure	page 8
III. DIC Splitting Procedure	page 18
IV. Cleaning Up	page 20
V. Before You Leave	page 20
VI. Cleaning Methods	page 21
VII. Operating Oven	page 22

Diagram

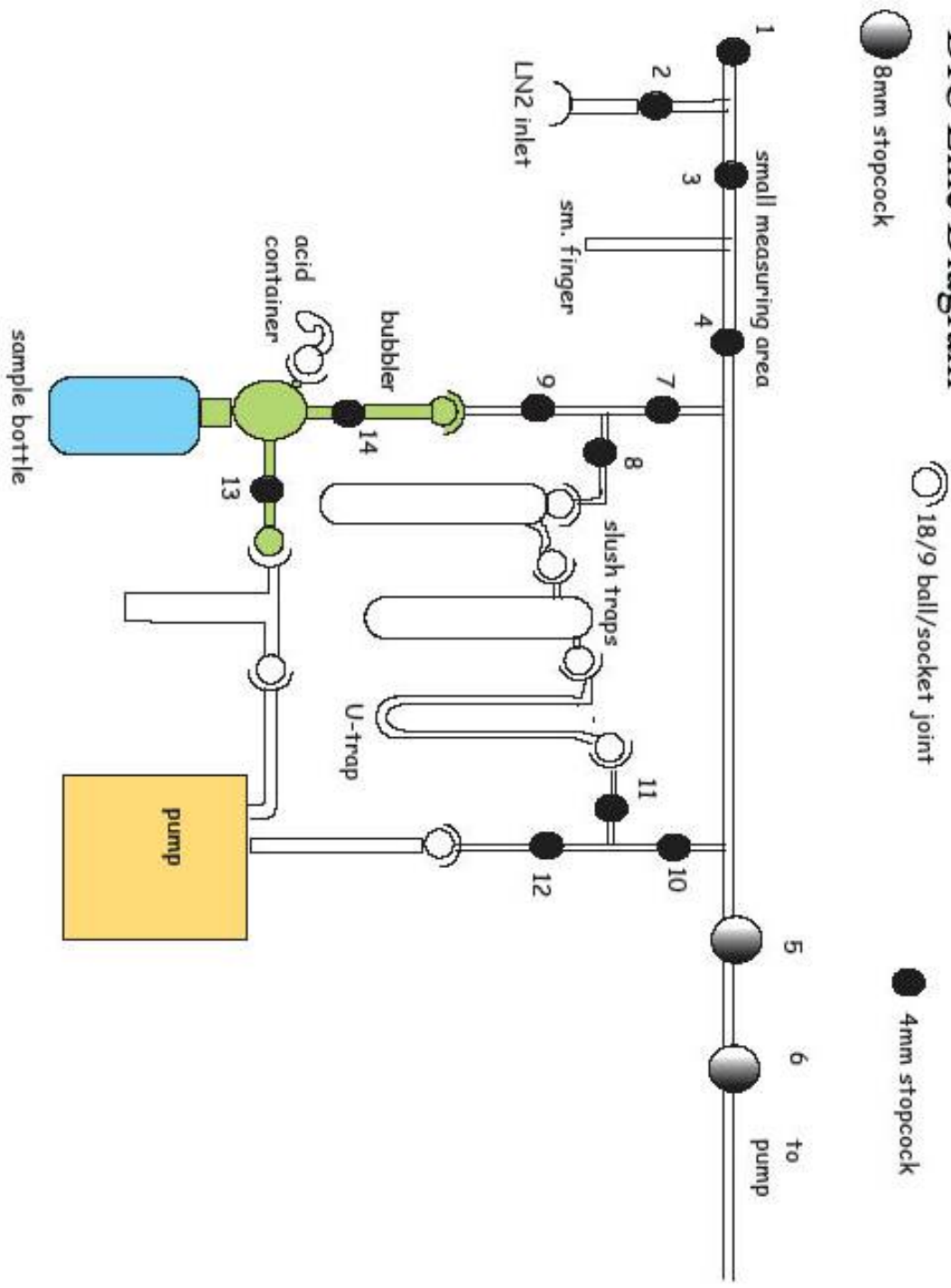
I DIC Line	page 4
------------	--------

List of Figures

Figure 1. Collecting Seawater	page 5
Figure 2. Thick Line of Apeizon Grease around Glass Stopper	page 6
Figure 3. Rubber Band Secured DIC Bottle	page 6
Figure 4. Thermometer in the collection bucket	page 7
Figure 5. The ice grinder	page 8
Figure 6. The oil traps	page 8
Figure 7. Water Traps on the line	page 9
Figure 8. Bubbler	page 10
Figure 9. N ₂ Gas tanks	page 10
Figure 10. Acid attached to bubbler	page 11
Figure 11. Glove Bag With Bubbler Set Up	page 12
Figure 12. Bubbler being attached to line	page 12
Figure 13. Line Inlet and Measure Volume	page 13
Figure 14. N ₂ Hose to Line Inlet	page 13
Figure 15. MKS Gauge	page 14
Figure 16. Recirculating Line Set Up	page 15

Figure 17.	MKS measure volume	page 16
Figure 18.	Slush around MKS measure volume	page 17
Figure 19.	Vacuum Line Attachments	page 18
Figure 20.	Tube Attached to Vacuum Line Attachment	page 18
Figure 21.	The Ovens	page 22
Figure 22.	The Timer Box	page 22
Figure 23.	The Oven Controls	page 23

DIC Line Diagram



I. Procedure to collect Seawater for DIC (Dissolved Inorganic Carbon), Salinity and Total CO₂ samples

Checklist for DIC sample collection

Cleaned DIC Bottle(s)	grease syringe (2cc glass tip syringe)
Cleaned Total CO ₂ Bottle(s)	sm. kimwipes
Cleaned Salinity Bottle(s)	Water Thermometer
Large Rubber Bands	Collection Notebook
Large Plastic Holding Crate	HgCl ₂ solution in a 50µl drop bottle
Collection Bucket with Tubing and Rope	grease, Apiezon N

1. Label the DIC, Salinity and Total CO₂ bottles twice with the date that the sample is being collected. Then record the labels and bottle #'s in the DIC collection notebook. This is much easier to do when the bottles are dry, so do it now before the bottle gets wet.
2. Lower the collection bucket from your sampling point and rinse the bucket 3 times with the seawater water. Once rinsed, fill the bucket as high as possible and quickly raise the bucket.



Figure 1. Collecting seawater with the bucket

3. To collect the DIC sample, rinse the reagent bottle at least 3 times, rinsing the ground glass stopper as well with the sample water.
4. Use a long piece of silicone tubing that is connected to the bucket (previously soaked in a 10% HCl solution) to fill the bottle until overflowing.
5. Put the stopper back into reagent bottle until ready to poison it.

6. After collecting the desired number of samples, prepare to POISON the DIC sample (this is to kill the organic compounds in the water sample so they will not alter the DIC concentration).
7. When ready to poison the sample remove the ground glass stopper.
8. Use a small kimwipe to dry the stopper and set it aside on clean surface (preferably cover the work area with a fresh new plastic).
9. Pour off some of the sample until the volume in the reagent bottle is about 5mm below the ground glass joint area.
10. Add 100 μ l of saturated HgCl₂ solution to each bottle (one drop from the little is equal to 50 μ l, so use two drops).
11. Dry the inside ground glass stopper with a kimwipe.
12. Put about two vertical strips of "N" grease (Apiezon N grease – it is important to use this brand because of its very low vapor pressure) on the dry glass stopper using the grease syringe. Figure 2. shows a good line of grease around the stopper.



Figure 2. Thick line of Apiezon N grease around the glass stopper



Figure 3. Rubber band secured DIC bottle

13. Place the stopper in the bottle. Twist and apply pressure to the stopper to insure a good seal. Shake the bottle well to mix in the HgCl₂. The mercuric chloride is a very poisonous substance that you do not want to get on yourself. Read the MSDS sheet for further safety info.
14. Secure the bottle top with two rubber bands as shown in figure 3. and place the bottle back in its plastic bag. Note that each reagent bottle has the same number etched into the stopper and the side of the jar. That will be your sample bottle number.

15. Using the same water used for the DIC sample, rinse the Total CO₂ bottle and cap 3 times with the water using the hose.
16. Fill the bottle until it is overflowing. Pour off some of the water so that it does not touch the stopper.
17. Add one drop of Mercuric Chloride (Hg₂Cl₂) poison to the bottle. Close the bottle and then shake a few times to thoroughly mix in the poison.
18. Rinse the Salinity bottle and cap 3 times with the same water from the bucket using the hose and then fill the Salinity bottle to about 1cm from the top of the bottle. **DO NOT POISON SALINITY SAMPLES.**
19. Dump the remaining water from the bucket at this point and collect another bucket of water. Immediately place the water thermometer in the bucket and wait at least 2 minutes before recording the temperature as shown in the following figure.



Figure 4. *Thermometer in the collection bucket*

20. Record the time, date, water temperature and how the bottles are labeled in the Collection notebook. Be sure to check the water temperature at least twice. Also, note the weather, sea state and any unusual conditions in the notebook. This will help later when analyzing the sample data.

II. DIC Extraction Procedure

1. Find the water traps and the CO₂ extraction bubbler and ensure they are clean and dry. All glassware should have been cleaned and baked out since the line was last used.
2. About 5-6 pounds of dry ice is necessary for the DIC extraction. This is typically enough to last all day. Once you have obtained the dry ice from the physical store you can grind it at the ice crusher in the lab.



Figure 5. The ice grinder

3. Place the empty dry large dewars on the 2 large LN₂ traps on the far right end of the DIC line. Secure the dewars. Use another LN₂ dewar to carefully fill the dewars to the top with liquid N₂ (L N₂). This will help you avoid burning your hand from the splashing LN₂ Open the line to the pump so it can start pumping down.



Figure 6. Oil traps

4. Make sure the O-rings are in good condition and place the two water and one CO₂ traps on the line. Clamp the traps to the line. Open the traps up to the vacuum because they will take a few minutes to pump down.

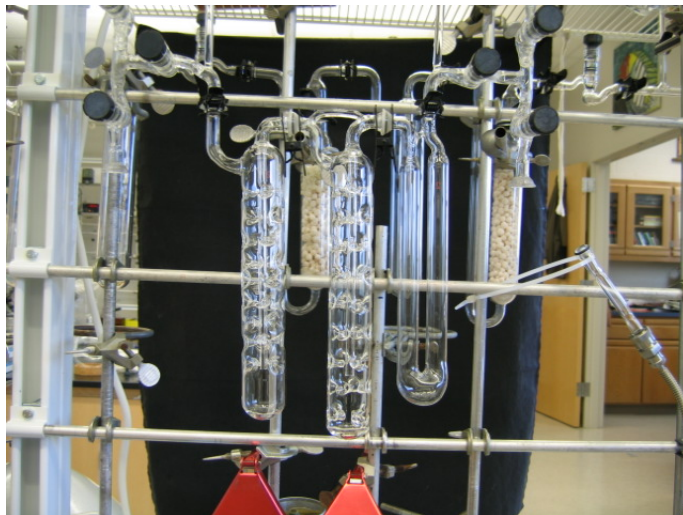


Figure 7. Water traps on line

5. While the traps are pumping down you can begin the assembly of the bubbler and get the DIC extraction notebook ready.
6. The DIC extraction notebook is in the drawer with the glassware. Fill the DIC extraction form out. Check with lab personnel about which UCID# to use. Record the UCID# into the DIC form along with all other pertinent information which includes date, user, bottle number, and so on. Leave the DIC binder on the counter to keep it accessible.
7. In the glove bag put:
 - Petri dish to hold O-rings, clamp, stopcocks
 - 2 O Rings (for 18/9 ball sockets)
 - 2 Stopcocks
 - 1 Clamp (for clamping acid containers)
 - 1 Paper Towel
 - Petri dish with a Syringe filled with sufficient Apeizon Grease
 - The Phosphoric Acid Container with 3ml (you must fill the container under the hood using gloves) and the weighed rings to stabilize the small beaker
 - Your weighed water sample (You must weigh your sample before with the rubber bands off)
 - 1 empty reagent bottle
 - 1 Bubbler



Figure 8. Bubbler

8. The N_2 gas tank is located at the left of the line as seen in the figure. Turn on the N_2 gas at a low flow to the glove bag. Squeeze the bag of all its air while being careful of the bubbler and the acid container. Use the rubber tubing in the glove bag to flush the air out of the acid container, reagent bottle, and bubbler. If the bag is filling too fast it may burst while you are preparing the slushes.



Figure 9. N_2 gas tanks

9. When the bag is filled to about $\frac{3}{4}$ full with N_2 you can compress the bag again with the clamp open to flush the bag. Altogether the bag must be flushed 3 times. It is also important to flush the inside of the bubbler, bottles and acid container with the hose to push out all the trapped air.
10. As previously mentioned, while the bag is filling you have some time on your hands so you should prepare the slushes. Place a small amount of isopropyl in 4 med. sized dewars. Then add small amounts of dry ice and mix. Continue the process until each dewar is about $\frac{2}{3}$ full with a fairly wet slush. If it is too dry it will not slide on to the water traps.

11. Once the bag has been flushed three times, you can fill it about $\frac{3}{4}$ full with gas and turn off the flow of N_2 gas. Too much N_2 gas in the bag will make maneuvering inside the bag difficult.
12. Assemble the bubbler. Then screw the stopcocks into the bubbler. Do not crank them real tight because like all the glass valves, they can break if they are screwed too tight.
13. Next, pour half of your weighed sample into the other bottle.
14. Apply more Apeizon grease to the ground glass stopper and seal your original sample. A good seal is necessary to store the bottle in case the you need to use the second half of the bottle.
15. Take your bubbler and apply a generous amount of grease to the bottom where it will fit into the bottle. However, no more than a couple thick lines of the expensive Apiezon grease are really necessary.
16. Once the grease has been applied to the bubbler you can fit the bubbler into the bottle containing your sample. Twist the bottle to ensure that there is an air tight seal.
17. Next take the acid container and apply a ring of grease around the top of the ball and clamp it to the bubbler. Make sure the acid container is positioned down when you attach it as seen in figure 10.



Figure 10. Acid container attached to bubbler

18. Finally, attach the O rings to the bubbler and carefully remove it from the glove bag.



Figure 11. Glove bag with Bubbler set up

19. Attach the bubbler to the line and pump out the area above the bubbler. Make sure the whole line up to the stopcock #3 is being pumped, excluding the bubbler itself.



Figure 12. Bubbler being attached to line

20. While the line is pumping, put the slushes on the first two of the water traps. Do not put anything on the 'U' trap yet.

Note: For the next part you must find lab personnel or someone else experienced with the DIC extraction procedure to supervise your first time because it is fairly difficult and easy to damage the line if the procedure is unclear to you.

21. Once the line has been pumped down and has reached its lowest vacuum, turn on the N₂ gas feed to the line at a fairly low flow. Put the tube up to the nitrogen inlet on the line and flush it thoroughly with N₂ gas three times. The finger flask for the sample will eventually attach to the nitrogen inlet socket joint.

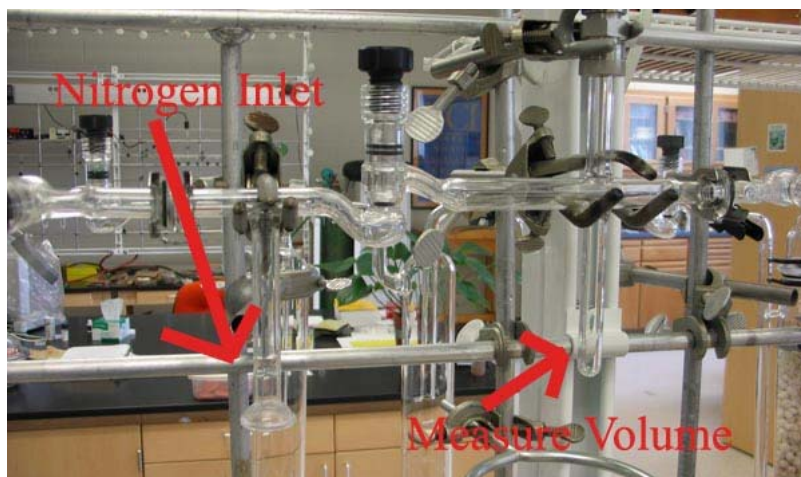


Figure 13. Line Inlet and Measure Volume

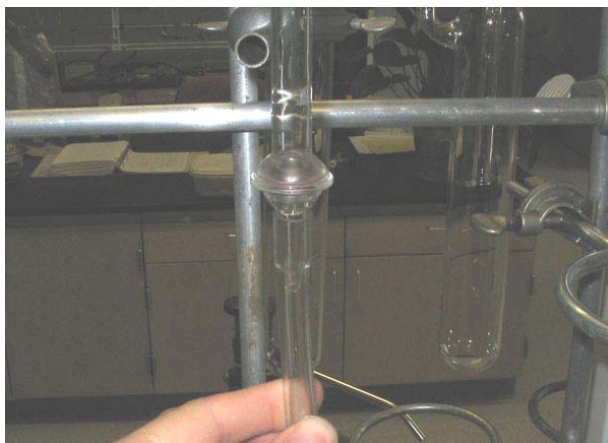


Figure 14. N₂ hose to line inlet

22. Make sure the stopcock (#5) connecting to the vacuum pump is closed and the area connecting the bubbler to the recirculating pump is not clamped. Open the stopcock (#3) between the N₂ inlet and measure volume. As gas volume increases in the line, open the stopcock (#12) above the recirculating pump. You should hear a noise coming from the connection of the bubbler to the circulating pump

when the pressure inside the line reaches about 80 cm Hg. (76 on the gauge is 1 atm). Allow the N₂ to flush through the line for three minutes.

23. Clamp the squeaking ball socket joint where the nitrogen is escaping with your fingers until the pressure reaches 100. Release the clamp, and repeat at least two more times.



Figure 15. MKS Gauge

24. Close the noisy connection with your fingers until the pressure reaches 100. Then release to help the line flush. Repeat this 2 more times.
25. This step must be done **quickly**. Hold the N₂ line into the inlet with your fingers and unclamp it. Keep holding the N₂ gas line to the inlet and clamp the ball socket joint that connects the bubbler to the recirculating pump. Close the stopcock (#3) from the N₂ inlet to the measure volume.
26. Close the stopcock (#12) to the recirculating pump from the water traps and place a dewar of liquid N₂ half way up on the 'U' trap. Allow the gauge to become steady.
27. Adjust the pressure of N₂ in the line to 53 on the MKS gauge. Slowly reduce the N₂ in the line from the large stopcock (#5) on the upper manifold that connects to the pump.
28. Once the pressure has reached 53 on the MKS gauge, close the large stopcock (#5). Open the top stopcock (#14) in the bubbler. Then open the bubbler stopcock to the recirculation pump. Record the line pressure into the DIC notebook.
29. Close both stopcocks (#7, #10) below the manifold, leading to the recirculating area. Open the stopcock (#13) on the bubbler leading to the recirculating pump. The recirculating area is now isolated from the line and the DIC stripping can begin.
30. Once again make sure all stopcocks in the recirculating portion of the line are open, plug in the circulating pump and pour the acid into the sample by twisting the acid container around. Set the timer for 10 minutes and record the start time in the notebook.



Figure 16. Recirculating Line Set Up

31. While the CO₂ is being stripped from the water you can do other things for your next sample.
32. Pump the finger flask on the N₂ inlet and pump out the N₂ from the rest of the manifold. Flame the finger flask out. This means to go over the glass with a 6 inch flame that is between blue and red. Bring the flame up and down the flask for no more than 10 seconds and let the flask cool. Flame the flask again after it cools. The finger flask can continue to pump until the sample is ready to be transferred into it.
33. Wash a bubbler from a previous extraction. Wear gloves and wipe the grease with a paper towel. The water in the bubbler is poisoned. Carefully dump the wastewater into the waste bucket. Wash the bubbler with soap and then rinse. Rinse with a 10% HCl solution and rinse with DI water at least three times. Let the bubbler dry on the rack and later bake it out in the oven at 550°C for two hours.
34. If you still have time you can get the glove bag ready for your next bubbler setup. Otherwise do this whenever you have a free moment.
35. You can also assemble dry water traps for your next extraction if you have time.
36. When 10 minutes are up, unplug the recirculating pump. Raise the liquid nitrogen dewar up on the 'U' trap and fill it all the way up. Set the timer for 2 minutes to allow any residual CO₂ to be frozen down.

37. Once 2 minutes has passed you can close the stopcocks (#14, #9) on the line above the bubbler and the stopcock (#12) above the recirculating pump. In addition, close the stopcock valve (#13) on the bubbler. Vent the recirculating pump's upper connection by removing the clamp beneath stopcock #12 and briefly remove the tube. Reclamp the area. The recirculating pump does not like air coming from the wrong direction so be careful. By venting the top first, air will only move the correct direction through the pump.
38. Carefully remove the bubbler.
39. Top off the liquid nitrogen in the dewar on the U shaped trap and slowly bleed the nitrogen from the line. Slowly bleed the nitrogen off using the stopcock (#5) on the upper manifold. Ensure that when the nitrogen is being pumped it does not cause the MKS gauge to go higher than 1.00. Allow the line to get pumped down to vacuum while continually topping off the liquid nitrogen.
40. Next you will transfer the CO₂ to the MKS gauge measuring area that is shown in figure 17. Place a small liquid nitrogen filled dewar on the tiny finger in the gauge area and close the line to the vacuum pump via the large stopcock (#5) on the upper manifold.

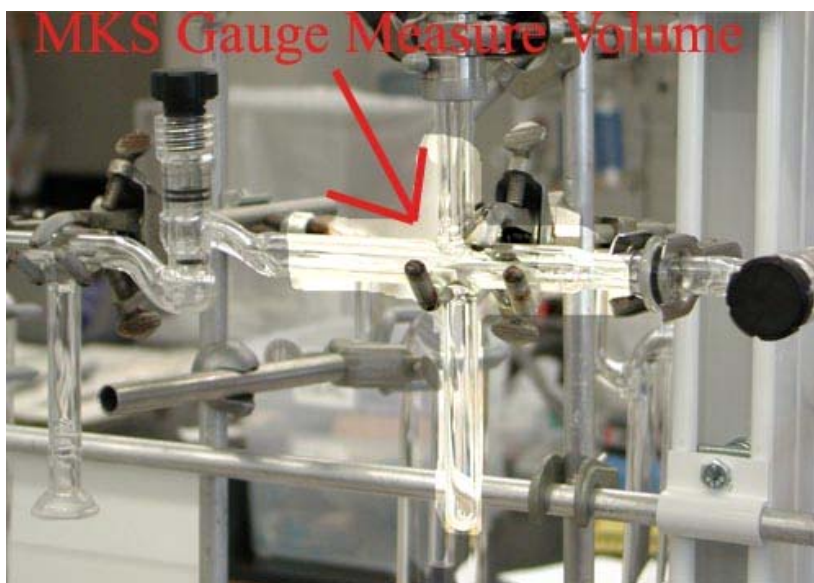


Figure 17. MKS Gauge Measure Volume

41. Quickly replace the liquid nitrogen dewar on the 'U' trap with a slush trap. This should cause the water to remain frozen in the 'U' trap while the CO₂ is transferred into the small finger on the measure volume. Set the timer for 10 minutes and allow the CO₂ to be transferred over. Continually top off the liquid nitrogen in the small dewar.

42. Once ten minutes has passed you can close the right side small stopcock (#5) on the main manifold. Pump sample area and manifold to highest vacuum. Close the stopcock (#4) to the right of the measuring area and remove liquid N₂ dewar. Let CO₂ expand in the measure volume.

43. You can now remove the water traps and place them in the sink.

Note: When you allow the gas to expand in the MKS gauge you may see some tiny water droplets. The water came from the water traps when the CO₂ was being transferred over. These cannot be allowed to remain with sample. The next steps will remove them from the rest of the CO₂ sample.

44. Take the other small dewar and make a slush.

45. Place this slush on the small finger of the measuring volume. Let expanded CO₂ sit in slush trap for three minutes to freeze down the water in the CO₂. Transfer your CO₂ over to the flamed out and labeled finger flask by placing a L N₂ dewar on the finger flask.



Figure 18. Small slush around measure volume

46. Once the CO₂ has been transferred over, close the stopcock (#3) to the left of the measuring area and pump out the water through the vacuum line.

47. Return the CO₂ in the finger flask to the small finger in the measuring area via a L N₂ dewar and expand the CO₂ again. Record the pressure in the DIC notebook.

48. If you are not planning on splitting the sample today, you can just transfer the CO₂ back to the finger flask via a L N₂ dewar. If you are planning on splitting the CO₂ you can continue on with the 'Splitting Protocol'.

Part III. Splitting Samples

1. Attach the finger flask the sample is contained in. If you are moving straight from the DIC extraction to the splitting, you will need to attach a finger flask anyways.
2. Take three baked sample tubes from their storage area. Ask lab personnel about where they are kept. Take one large tube for the excess CO_2 , one medium size for the C^{14} measurement, and one small for the C^{13} measurement.
3. Label each tube with the UCID number twice with an industrial permanent marker. Writing legibly will save time and confusion in the future.
4. Attach each tube to the “vacuum line tube attachment” and clamp them to the splitting manifold in the line with the metal clamps. There are three sizes of the attachments as seen in the figure, one for each type of tube. It is easiest to place the excess tube in the back of the other two tubes.



Figure 19. Vacuum Line Attachments



Figure 20. Tube Attached to Vacuum Line Attachment

5. Once all the tubes and the finger flask have been attached, evacuate the line through the bypass.
6. Flame the sample tubes out once they have reached vacuum. Wait until the gauge is as low as possible before closing the stopcock from above the finger flask to the rest of line.

7. Transfer the CO₂ from the finger flask to the measure volume via a liquid nitrogen dewar and expand it to room temperature. Record the gauge number in the notebook.
8. Transfer approximately 2.9-3.5 from the measure volume to the area above the finger flask. This is by far the most frustrating part of the procedure so you may have to refreeze down the CO₂ a number of times before you transfer between 2.9 and 3.5 to the measure volume. Ensure the finger flask has been closed before you transfer the CO₂ into the area above it.
9. Transfer the CO₂ into the C¹⁴ measurement tube using a liquid nitrogen dewar around the measurement tube. When the sample has been frozen down, vacuum out the noncondensibles. Once the gauge has reached a minimum you can close the tube to the vacuum and open the next tube to the vacuum so that it will be ready for your next sample.
10. Flame the next tube out in the sequence before sealing the C¹⁴ tube.
11. Seal the C¹⁴ tube with the torch. Keep the flame moving up and down as well as side to side. This takes practice so ask a lab personnel to supervise your first few times.
12. Take the exact number of the CO₂ transferred from the gauge down and multiply it by 41.1 then divide it by 76 to get the measurement into ml. Record that number down in the DIC notebook
13. Once the C¹⁴ tube has been sealed, vacuum out the area above the finger flask again. Then transfer 1.0-1.4 into the area above the finger flask. Transfer the CO₂ into the C¹³ tube. Vacuum out the noncondensibles, close it to the vacuum and seal the tube. Flame the excess tube out.
14. Now you will record the number of ml into the notebook and transfer the rest of the CO₂ into the excess tube. Make sure to record how much CO₂ is being transferred into the excess tube.
15. Congratulations you are done splitting that sample! Continue splitting until you are out of time. Each sample should take about 30mins to an hour plus to split. If you are spending more than this it would be best to ask a qualified lab personnel person to help your splitting technique.

Part IV. Cleaning Up (you must clean up):

- USE GLOVES WHEN HANDLING ACID OR ACETONE.
- All Bubblers, Water Traps, Bottles and Acid Containers must be wiped free of the Apeizon Grease.
- Wipe the gloves of the Apeizon Grease because the stuff is very tough to get off.
- After the Apeizon Grease has been wiped off, wash the glassware with soap. Then rinse with hot water and then wash the item with 10% HCL. Finally wash the item with distilled water and let dry on the rack. The water traps only need to be dry yet the bubblers should be baked out at 200°C before re-use.
- If there is grease still on the glassware it will show up when the item is dried or baked at 200°C in the oven.
- If you have gotten all the grease off the glassware that was possible to remove by hand, you can still bake the item out at 500°C in the oven for a few hours. See oven operation section for further instruction for operating the oven.
- NOTE: The new oven is very complex so do not use it, instructions for using the older oven are in “Part VII. Operating the Oven”.

Part V. Before you leave:

- Vent the regulators on the N₂, O₂ and gas.
- Close all stopcocks on the line.
- Put everything back in its proper place and remove the large L N₂ dewars from the line.
- Make sure water traps will be ready for the next day.

Part VI. Cleaning Methods

1. CO₂ Bottles

1. Separate Caps
2. Wash bottles and caps in a weak soap solutions
3. Rinse very well with tap water
4. Rinse with a 10% HCl solution
5. Rinse 3 times with distilled water
6. Bake in a muffled furnace at 550°C for two hours
7. When bottles are dry, remove from oven, cap and number them

2. Glass Sample Jars

1. Wash with soap and water new jars, otherwise start soaking
2. Rinse jars in 10% HCl
3. Rinse 3 times with distilled water
4. Put jars on steel sheets, cover lightly with cleaned Al foil
5. Bake in muffle furnace at 550°C for two hour
6. When cooled, cap jars
7. Put Jar in an appropriate sized baggie

3. Plastic Caps

1. Carefully remove the plastic inserts so they do not get damaged
2. Wash caps and inserts in a weak soap solution
3. Rinse very well
4. Rinse with a 10% HCl solution
5. Rinse 3 times with DI water
6. Dry, lightly covered in hood
7. Assemble the caps and cover the cleaned glass sample jars

Part VII. Operating the Oven

1. The oven to use is the brown one on the right as seen in the figure 21. It is the older rusted looking oven. The newer oven is very complicated to use and it is better to ask lab personnel if it is absolutely necessary to use the newer oven.



Figure 21. The ovens

2. Load the glassware in a position that will be steady.
3. Begin by setting the timer box on top of the oven. The timer box supplies power to the oven. It needs to be set for a bit longer than the time your glassware needs to be baked to allow for warming up to temperature time. For a 550°C bakeout set the oven for 4 hours. Two hours to warm up and two hours at temperature.



Figure 22. The Timer Box

4. Set the oven by pressing the “Press to Set” button and then turning the “Set Point” dial until the desired temperature is reached. Tighten the ring around the “set point” dial.



Figure 23. The Oven Controls

Note: Pyrex should not be baked above 550°C, otherwise it will melt

5. At this point the oven will begin heating up to the set temperature and stay at that temperature until the time on the timer box runs out.