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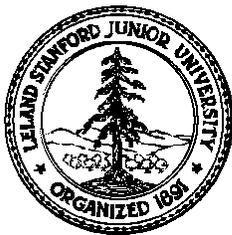
Stable Isotope Laboratory

Standard Operating Procedures Manual

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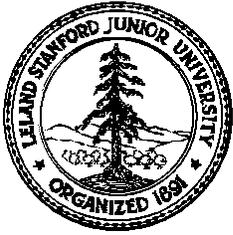




Stanford University Stable Isotope Lab
On-line Manual
(Prepared by David A. Mucciarone)

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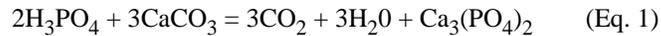


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Section 1A: Finnigan MAT252 with Kiel III carbonate device

I) Operation Principles for $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$ Analyses in carbonates:

Before you can operate the Finnigan MAT252 mass spectrometer, you must have training. Once trained, the following procedures will serve as an operational guide. The Kiel III carbonate device (KCIII) is connected to the MAT252 mass spectrometer changeover valve via a capillary. To get an understanding on how this process works, I will use calcium carbonate (CaCO_3) as an example and go from reaction of this material to mass spectrometer analysis. When CaCO_3 is reacted with purified phosphoric acid, at 70°C in our lab, CO_2 and H_2O gas are produced (Eq. 1).



The water is removed by a method called fractional freezing. Since water has a freezing temperature warmer than CO_2 the traps on the KCIII can heat or cool the trap as needed to control the transfer of CO_2 . The KCIII works in the following manner (see Fig 1). The sample is acidified with two drops of purified phosphoric acid. Both CO_2 and H_2O are frozen into Trap 1 at -196°C . The reaction time is 470 seconds. At the end of the reaction, non-condensable gases are pumped away for 30 seconds. Trap 1 is then warmed to -115°C to keep the H_2O frozen but allows the CO_2 to evolve as a gas. Trap 2 is then cooled to -196°C to trap

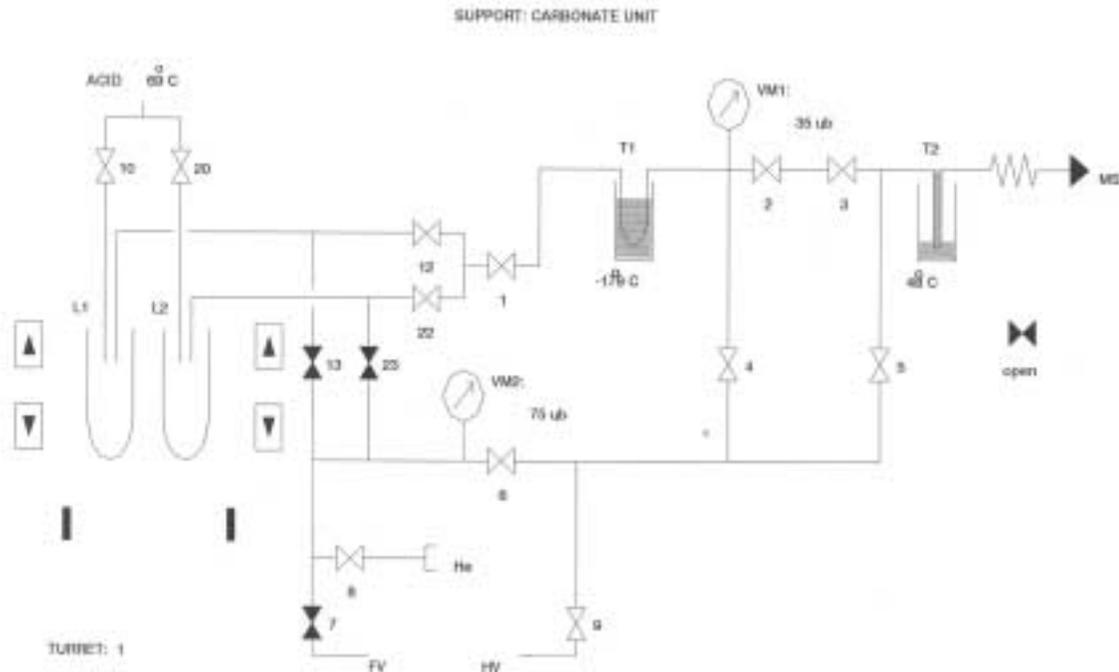


Figure 1: Schematic diagram of Kiel III carbonate device. The numbers denote valve identification, T1 and T2 represent Traps 1 and 2 respectively. HV represents high vacuum, which includes roughing and 60L turbo pump, FV is for fore vacuum which only has a roughing pump. Turret # indicates position of carousel. VM1 and 2 monitor vacuum integrity. MS indicates capillary attaching Kiel III carbonated device to changeover valve on the MAT252 mass spectrometer.

the CO₂ gas. After 120 seconds at -196°C, Trap 2 is isolated from Trap 1 by a series of valves. Trap 2 is then warmed to 30°C and the resulting CO₂ then travels down the capillary to the changeover valve.

Attached to the other side of the changeover valve is a capillary connected to the mass spectrometer inlet system (Fig 2). The inlet system is a symmetrical valve system composed of a reference and sample side where the valve volume and configuration are identical on both sides. Also equipped on

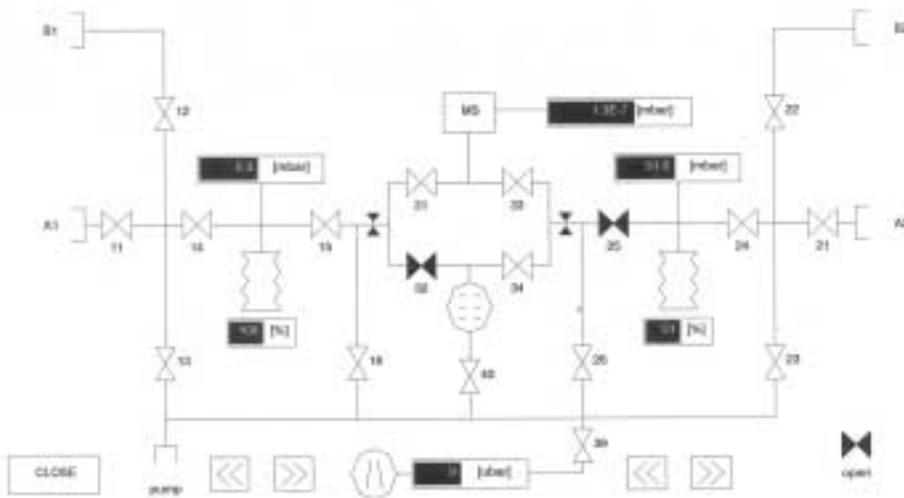


Figure 2: Schematic diagram of MAT252 inlet system. The numbers denote valve identification, valve numbers 31 - 34 represent the changeover valve. Volume of inlet bellows are indicated in %, inlet gas pressures are measured in mbar and roughing vacuum in μ bar. MS indicates capillary attaching the inlet system to changeover valve on the MAT252 mass spectrometer.

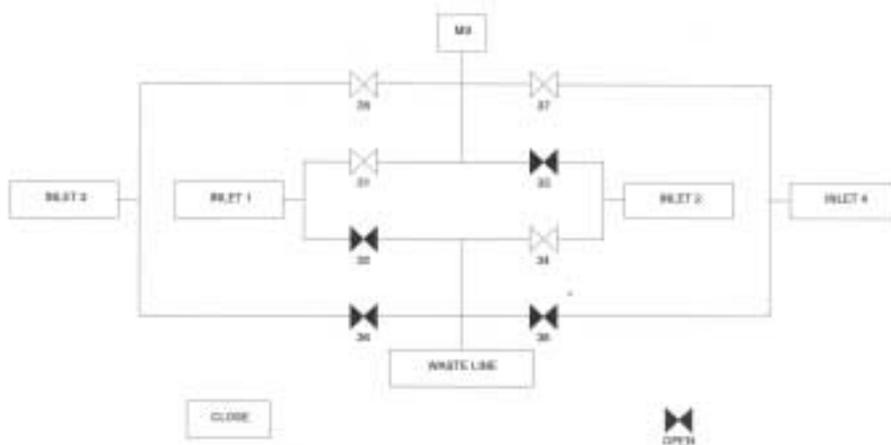


Figure 3: Schematic diagram of MAT252 changeover valve. Inlet 1 connects to the left side (sample) of inlet system, Inlet 2 connects to the right side (reference) of the inlet system, Inlet 3 connects to the Kiel III carbonate device, and Inlet 4 is a spare. MS indicates capillary attaching the inlet system to changeover valve on the MAT252 mass spectrometer.

the inlet system are variable reservoirs (flexible bellows) which allow for additional control of inlet gas pressures. A reference CO₂ gas (gas of known isotopic composition) is placed into the reference side of the inlet system for comparison with a known or unknown sample gas from the carbonate device. A series of standards of known isotopic composition are commonly run through the carbonate device to assure proper reference gas calibration and sampling protocol. The reference gas is a large quantity of gas that occupies the entire reference side of the inlet including the bellows. This gas will remain in the inlet throughout many known and unknown sample runs. In order to obtain useful isotopic data it is necessary to balance the pressure and volume of the known (reference gas) and unknown (sample from carbonate device) gas. This is done using the reference bellows and valves in the inlet system. Gas in the inlet system (Reference side) and carbonate device flow down capillaries and through changeover valves (Fig 3) into the ion source. These capillaries have an extremely small inside diameter. At the end of the capillaries are crimps, which are adjusted so that the flow and depletion rates of sample and reference CO₂ gas are identical. The crimps are also the transition point between low vacuum viscous (< 10⁻⁴ Torr) and high vacuum molecular (>10⁻⁴ Torr) gas flow. The capillaries are connected to a set of changeover valves that control the input of the inlet gases allowing for only one gas to be analyzed at a time.

Once the gas passes through the changeover valves it enter the source through an inlet probe (not to be confused with the inlet system) which simply directs the gas flow directly into the source block, more precisely the ionization chamber (see Fig 4). The gas flow in the ion source is always molecular. Attached to the source block are the filament (cathode), trap, ion extraction plates, and focusing plates (Fig. 5).

- a) The filament (cathode), often tungsten, emits electrons in a helical orbit under the influence of small source magnets in which the CO₂ gas molecules collide with the electron beam making a CO₂⁺ ion. The electron beam is at right angles to the ion trajectory.
- b) The trap collects all electrons not involved in the collision with the CO₂, and is positioned opposite the filament.
- c) The extraction plates accelerate ions out of the ionization chamber and direct the ions toward the exit slits and focusing plates. The accelerating voltage on the MAT252 is 10kV.

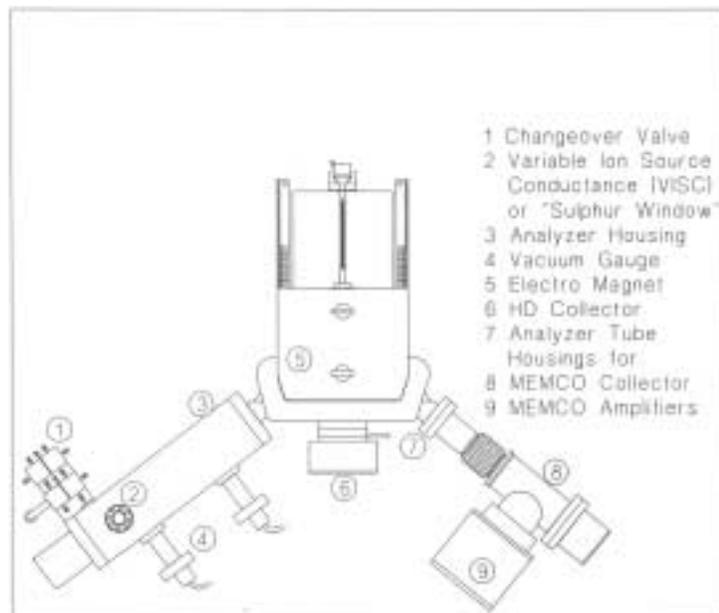


Figure 4: Schematic representation of the MAT252 analyzer system and the arrangement of components. Illustration is from the Finnigan operations manual.

- d) There are a number of different focusing plates that are designed to progressively focus the ion beam leaving the source. The voltage is variable depending on the isotopic species being analyzed.
- e) The final focusing plate defines the maximum beam width prior to passage into the flight tube.

The flight tube has a 90° deflection path (Fig. 4) having a radius of R fitted with a permanent magnet of strength B . The MAT252 is equipped with an electromagnet with a maximum field strength of 0.77 Tesla providing a mass range up to 150 amu at full accelerating voltage. Therefore the radius is fixed and the magnet is variable but constant for any given gas species. At a constant accelerating voltage (V) and magnet strength, the lighter mass will be deflected (bend) more than the heavier mass. In the case of CO₂, mass 44 is bent more than 45 and 46. However, in order to analyze another gas such as N₂ or SO₂ with a fixed magnet, the accelerating voltage needs to increase or decrease, respectively or the accelerating voltage can remain the same and the magnetic field strength can be varied. Therefore, the relation of all these parameters is given as:

$$m/z = K * H^2$$

Where: m/z is the mass to charge ratio
 $K = R^2/2U = \text{constant}$
 H is the magnet strength
 R is the Radius of flight tube
 U is the acceleration voltage

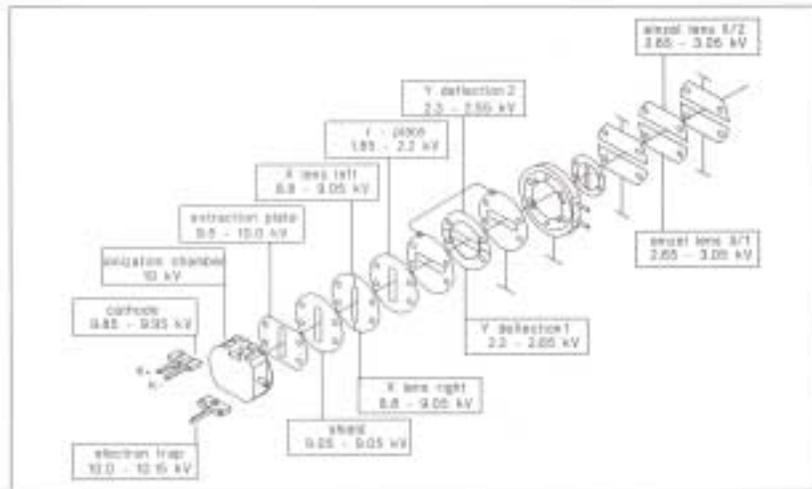


Figure 5: Schematic of MAT252 ion source. Values are ion source potential in relation to ground at 10kV ion acceleration voltage.

After leaving the flight tube, the separate ion beams of different masses are directed into 2 (1 major and 1 minor) or 3 (1 major and 2 minor) collectors (Faraday cups) depending on the type of mass spectrometer (Fig. 6). These cups are grounded through high ohm resistors. As the ion current passes to ground the drop in the resistor acts as a measure of the ion current. There are two types of collectors, major and minor. The major collector is larger and less sensitive than the minor collector. These collectors can either be fixed to a correct spacing for a particular set of masses or adjustable. In the case of CO₂, a triple collector mass spectrometer can detect all three masses (44-major, 45-minor, and 46-minor) and calculate δ¹⁸O and δ¹³C in one run. On a double collector with CO₂ the accelerating voltage is changed to direct the appropriate masses into the 2 collectors in order to calculate δ¹⁸O and δ¹³C (44-major, 45-minor for δ¹³C, 44 & 45-major, 46-minor for δ¹⁸O).

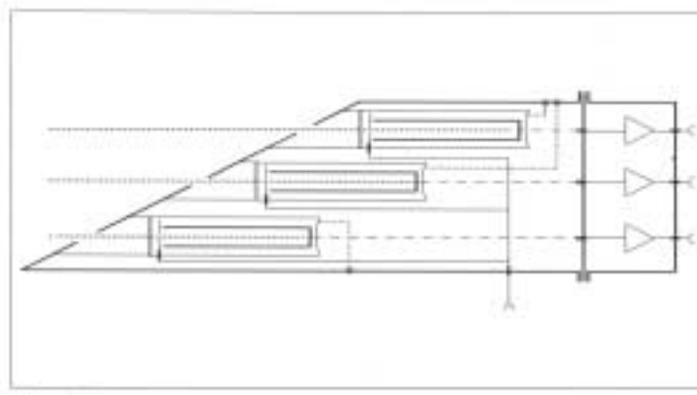


Figure 6: Schematic representation of faraday cup (collectors) on the MAT252.

The signals received by the collectors are amplified and transferred to voltage to frequency converters (VFC) on a system control board in a microprocessor. This system board basically digitizes the analog signal for processing by an interfaced computer. The analog and digital output is then sent to disk and a printer. In the course of one analysis for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ on the MAT252 mass spectrometer, 8 reference/sample/reference sets (8 traces or 24 changeover valve cycles) are analyzed. All of the data are expressed in conventional delta (δ) notation, where the isotopic ratios of $\delta^{18}\text{O}/\delta^{16}\text{O}$ and $\delta^{13}\text{C}/\delta^{12}\text{C}$ are relative to the international PDB standard (Eq. 2) as defined by:

$$\delta \text{‰} = [(\text{Ratio}_{\text{sample}} - \text{Ratio}_{\text{standard}})/(\text{Ratio}_{\text{standard}})] \times 1000 \quad (\text{Eq. 2})$$

PDB refers to a Cretaceous belemnite called *Belemnitella americana* from the Peedee formation in South Carolina and is defined as 0 for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and has been exhausted (however, there may be a few labs that still have a small quantity remaining). The carbonate standard NBS-19 or TS limestone (NBS is an acronym for the National Bureau of Standards which was replaced by NIST) is presently a substitute for PDB and has been given a fixed isotopic value for $\delta^{13}\text{C}$ of 1.95‰ and for $\delta^{18}\text{O}$ of -2.2‰. Many laboratories use internationally known standards such as NBS-19 obtained from facilities such as the National Institute of Standards and Technologies (NIST) or from the International Atomic Energy Agency (IAEA) to calibrate their instruments and reference gases. To reduce the possibility of exhausting these international standards, laboratories establish working standards that have been calibrated against various known standards and with other laboratories, to use routinely in the laboratory.

Although PDB is always used for reporting $\delta^{13}\text{C}$ results, in addition to PDB, the term SMOW is also used when referring to $\delta^{18}\text{O}$ in carbonates and water. SMOW is an acronym for Standard Mean Ocean Water introduced by H. Craig (1961b). Like PDB, SMOW is defined as 0 for $\delta^{18}\text{O}$, however, SMOW does not equal PDB. To convert PDB to SMOW or vice versa Friedman and O'Neil (1977) generated the following equations.

$$\delta^{18}\text{O}_{(\text{SMOW})} = 1.03091(\delta^{18}\text{O}_{(\text{PDB})}) + 30.91 \quad (\text{Eq. 3})$$

or

$$\delta^{18}\text{O}_{(\text{PDB})} = 0.97002(\delta^{18}\text{O}_{(\text{SMOW})}) + 29.98 \quad (\text{Eq. 4})$$

You may also find this standard referred to as Vienna-Standard Mean Ocean Water or V-SMOW which was prepared by H. Craig for the IAEA in Vienna, Austria (Hoefs, 1980). Two other water standards used are SLAP (Standard Light Antarctic Precipitation) which was introduced by Gonfiantini in 1976

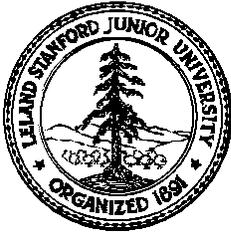
(Gonfiantini, 1978) for the IAEA, and GISP (Greenland Ice Sheet Precipitation) which was prepared by W. Dansgaard. Listed in Table 1 are the isotopic compositions of commonly used standards, most of these can be obtained from the National Institute of Standards and Technology (NIST) in Gaithersburg, MD 20899-0001. For more information consult the reference list below.

Table 1: Isotopic compositions of standard reference materials.

Standard.	Description (‰)	$\delta^{18}\text{O}_{(\text{PDB})}$ (‰)	$\delta^{18}\text{O}_{(\text{SMOW})}$ (‰)	$\delta^{13}\text{C}_{(\text{PDB})}$ (‰)
PDB	Peedee belemnite	0.00		0.00
SMOW	Standard Mean Ocean Water		0.00	
NBS-1	Water		-7.86	
NBS-1A	Water		-24.21	
NBS-18	Carbonatite	-23.05		-5.04
NBS-19	TS limestone	-2.20	28.65	1.95
NBS-20	Solenhofen limestone	-4.18	26.64	-1.06
NBS-21	Graphite			-28.10
NBS-22	Oil			-29.63
SLAP	Standard Light Antarctic Precipitation		-55.50	
GISP	Greenland Ice Sheet Precipitation		-24.85	

II) References:

- Coplen, T.B., C. Kendall, J. Hopple, 1983. Comparison of stable isotope reference samples, *Nature*, 302:236-238.
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- Epstein, S., R. Buchsbaum, H. Lowenstam, and H.C. Urey, 1953. Carbonate-water isotopic temperature scale, *Geol. Soc. Am. Bull.*, 62:417-426.
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- McCrea, J.M., 1950. On the isotopic chemistry of carbonates and a paleotemperature scale, *J. Chem. Phys.*, 18:849-857.
- Shama, T., and R.N. Clayton, 1965. Measurement of $^{18}\text{O}/^{16}\text{O}$ ratios in total oxygen of carbonates, *Geochim. Cosmochim. Acta*, 29:1347-1353.
- Swart, P.K., S.J. Burns, and J.J. Leder, 1991. Fractionation of the stable isotopes of oxygen and carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function of temperature and technique, *Chem. Geology*, 86:89-96.
- Walters Jr., L.J., G.E. Claypool, and P.W. Choquette, 1972. Reaction rates and $\delta^{18}\text{O}$ variation for the carbonate-phosphoric acid preparation method, *Geochim. Cosmochim. Acta*, 36:129-140.



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Section 1B: Finnigan MAT252/Kiel III Carbonate Device Operation

I) Run terminated without error (NORMAL)

1. Click on ALT-T (upper left screen). This will exit CNF-B, ACON-B
2. Click on CNF-B, SUPP-B. This will enter Kiel support screen
3. Click on TAKE MAG (F7). This will remove vials from ports (Fig. P1).
4. Lower and remove liquid nitrogen (LN₂) dewar and remove ice (Fig. P2). This is necessary if beginning a new run or if run terminated by error and was sitting many hours.
5. Remove carousel (Fig. P3). Be careful not to hit capillaries or drip counter.
6. Install new carousel (Fig. P3), again be careful not to hit the drip counters or capillaries.
7. Clean acid drip counter, and capillaries with 2-propanol (Fig. P4).
8. Clean o-rings on both ports with 2-propanol and then lightly grease o-rings with Apiezon L (Fig. P5).
9. Click on KBD INPUT (F5) Type P5 <Enter>, Click on KBD INPUT again Type P1 <Enter> this will reset the carousel.
10. If ice around cold traps has been removed and LN₂ is ready (*i.e.*, LS160 dewars and small blue dewar replaced and approximately 1" from top), click on T1 then click on analog temperature bar at approximately -170°C to -180°C. This will fill the blue LN₂ dewar. Remember as it begins to fill, approximately 1/2, bring the dewar snug to the cold trap plate.
11. If LN₂ is not ready, click on LOAD MAG (F6). This will load the vials on the ports (Fig. P6).
12. Click on CNF-B, EDIT-B, 2-SEQUENCE EDITOR. Prepare a new table by copying a previous sequence. **The filename should be in the format operators first and last initial followed by the date (e.g., DM041902).** Enter new run information. Make sure that the reference refills (RR=120) are in the correct locations. When finished, save and exit Sequence Editor, then Click on ALT-T.
13. Return to CNF-B, SUPP-B, if acid temperature is at 69°C and LN₂ dewar is filled and cold trap is at the temperature you selected above then Click ALT-T.
14. Click on CNF-B, ACON-B, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: (*e.g.*, DM041702)
 - b. START LINE: 1,
 - c. END LINE: 46, make sure start and end line numbers are correct (*e.g.*, 1 and 46 for a full carousel run)
 - d. MODE: DAY
 - e. FILENAME: **should be as follows: Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format operators first initial, date, followed by a letter beginning with A ending with .WKS (e.g., D041702A.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D041702A.WKS).
 - f. LIST TYPE: FORMAT3 (ONLY)
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: COL
15. Click SAVE to save sequence.
16. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D041700A.WKS A:). Press Ctrl and 1 (from the number keypad) to return to ISODAT.
17. Click MEASURE to begin run. Make sure there is plenty of paper in printer to complete the run.



Stanford University Stable Isotope Laboratory Finnigan MAT252 mass spectrometer with 24 port manifold, and Kiel II carbonate device.

Stanford University MAT252/Kiel III Carbonate Device Operation

II) Run terminated with error

1. Click on ALT-T (upper left screen). This will exit CNF-B, ACON-B
2. Click on CNF-B, SUPP-B, 1-CARBONATE UNIT. This will enter Kiel support screen
3. Remove liquid nitrogen (LN₂) dewar and remove ice (Fig. P2). This is necessary if run was terminated by error and was sitting many hours.
4. If ice around cold traps has been removed and LN₂ is ready (*i.e.*, LS160 dewars and small blue dewar replaced and small blue dewar is approximately 1" from top), click on T1 then click on analog temperature bar at approximately -170°C to -180°C. This will fill the blue LN₂ dewar. Remember as it begins to fill, approximately 1/2, bring the and small blue dewar snug to the cold trap plate.
5. Click on CNF-B, EDIT-B, 2-SEQUENCE EDITOR. Select table that was terminated by error and add and/or delete reference refills (RR=120) and make sure that the RR=120 are in the correct locations. When finished, save and exit Sequence Editor, then Click on ALT-T.
6. Return to CNF-B, SUPP-B, if acid temperature is at 69°C and LN₂ dewar is filled and cold trap is at the temperature you selected above then Click ALT-T
7. Click on CNF-B, ACON-B, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: reselect the correct table name (*e.g.*, DM041702)
 - b. START LINE: begin where the error took place
 - c. END LINE: 46 if for a full carousel run
 - d. MODE: NIGHT
 - e. FILENAME: should be as follows: **Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format, operators first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D041702B.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D041702B.WKS).
 - f. LIST TYPE: FORMAT3 (ONLY)
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: COL
8. Click SAVE to save sequence. Make sure there is plenty of paper in printer to complete the run.
9. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D041702A.WKS A: or COPY D041702*.*: A to copy all associated files). Press Ctrl and 1 (from keypad) to return to ISODAT.
10. Click MEASURE to begin run.

Stanford University MAT252/Kiel III Carbonate Device Operation

III) Run interrupted by error

1) PART 1: Able to escape out of error

- A. If a gray window with a red bar will be displayed on the screen obstructing the normal display. Locate the source of the error (*e.g.*, high voltage out). Fix error then attempt to press ESC. If the error display disappears and the instrument appears to be working watch it carefully because it may actually be hung up and just going through the motions. It is best to just press TERMINATE and allow the sample that is in acquisition to finish. If the error appeared a short time ago it is just a simple matter of restarting the sequence as follows
1. Click on ALT-T (upper left screen). This will exit CNF-B, ACON-B.
 2. Click on CNF-B, ACON-B, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: reselect the correct table name (*e.g.*, DM041702).
 - b. START LINE: begin where the error took place.
 - c. END LINE: 46 if for a full carousel run).
 - d. MODE: DAY
 - e. FILENAME: should be as follows: **Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format, operators first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D041702B.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D041702B.WKS).
 - f. LIST TYPE: FORMAT3 (**ONLY**)
 - g. FILE TYPE: LOT
 - h. SAVE DATA to database: COL
 3. Click SAVE to save sequence. Make sure there is plenty of paper in printer to complete the run.
 4. Click MEASURE to begin run.
 5. If you were able to escape from the error or the run was able to continue the data was saved to the database (called ISOBASE) and to the filename you created. On the other hand, if you were not able to escape and had to refer to Part 2 of this section then the data was only saved to the database and not to the file you created in the SEQUENCE ACQ screen. This file will only have 128K. You will need to review the recover data section (IV) for instructions on how to obtain the run data.

Stanford University MAT252/Kiel III Carbonate Device Operation

2) PART 2: Unable to escape out or error

- A. If a gray window with a red bar will be displayed on the screen obstructing the normal display. If after looking for the fault and do not see one then attempt to press ESC. If the error display does not disappear to the point where the screen freezes then you will have to reboot the computer by following the reboot sequence.
1. Press Ctrl, Alt, Del to reboot computer.
 2. At the Load Real/32 prompt, Type N <Enter> this will load Win95
 3. At login prompt, Type sil <OK>
 4. Click on Scan Disk (on desktop). Let the program delete any fragments - DO NOT FIX THEM, just delete them.
 5. Restart the computer using the START button
 6. At the Load Real/32 prompt, Type Y <Enter> this will load ISODAT v7.2 allow the microprocessor to reset before doing anything (message is displayed at top of screen).
- B. Continue as follows to prepare the run:
1. Click on CNF-B, SUPP-B, 1-CARBONATE UNIT. This will enter Kiel support screen.
 2. Depending on where the carousel stopped rotating (check to see its present position) you will have to enter this value because the **Turret position will default to 0 (zero)**. This must be reset. (*e.g.*, the carousel failed at position 5. Using KBD INPUT Type P5, then Type P1. The carousel will not rotate at the first input but will rotate to position 1 on the second. If the carousel failed at position 1 then you only need to Type P1, the carousel will not rotate but the turret will be reset.
 3. Remove liquid nitrogen (LN₂) dewar and remove ice. This is necessary if run was terminated by error and was sitting many hours (Fig. P2).
 4. If ice around cold traps has been removed and LN₂ is ready (*i.e.*, LS160 dewars and small blue dewar replaced and small blue dewar is approximately 1" from top) Click on T1 then Click on analog temperature bar at approximately -170°C to -180°C. This will fill the blue LN₂ dewar. Remember as it begins to fill, approximately 1/2, bring the and small blue dewar snug to the cold trap plate.
 5. Click on CNF-B, EDIT-B, 2-SEQUENCE EDITOR. Select table that was terminated by error and add and/or delete reference refills (RR=120) and make sure that the RR=120 are in the correct locations. When finished, save and exit Sequence Editor, then Click on ALT-T.
 6. Return to CNF-B, SUPP-B, if acid temperature is at 69°C or 70°C and LN₂ dewar is filled and cold trap is at the temperature you selected above then Click ALT-T

Stanford University MAT252/Kiel III Carbonate Device Operation

2) PART 2: Unable to escape out or error (CONTINUED)

7. Click on CNF-B, ACON-B, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: reselect the correct table name (*e.g.*, DM041702).
 - b. START LINE: begin where the error took place.
 - c. END LINE: 46 if for a full carousel run).
 - d. MODE: DAY
 - e. FILENAME: should be as follows: **Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format, operators first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D041702B.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D041702B.WKS).
 - f. LIST TYPE: FORMAT3 (**ONLY**)
 - g. FILE TYPE: LOT
 - h. SAVE DATA to database: COL
8. Click SAVE to save sequence. Make sure there is plenty of paper in printer to complete the run.
9. You need to remember that when a run is terminated by error in this way the data is saved to the database (called Isobase) only and not to the file you created in the SEQUENCE ACQ screen. This file will only have 128K. You will need to review the recover data section (IV) for instructions on how to obtain the run data.
10. Click MEASURE to begin run.

Stanford University MAT252/Kiel III Carbonate Device Operation

IV) Recover run data from database

1. Click on CNF-A, EVAL-A, 1-OFF LINE
2. (6) SEQUENCE NAME: (*e.g.*, DM041702) Search by sequence name of run.
3. Click on DIRECTORY (Shift F5).
4. Click on TAG ALL (F5) if all of the runs need to be transferred to disk or use TAG (F6) to select the runs needed to be transferred.
5. Click on FORMAT (F8) and then enter the following exactly:
 - a. OUTLIER TEST: D2
 - b. STANDARD IDENT: SIL#2
 - c. LIST TYPE: NO
 - d. GRAPHICS: NO
 - e. ALPHA (CO3) CORRECTION: YES
 - f. ALPHA (H2O) CORRECTION: NO
 - g. ATOM% / APE REPORT: NO
 - h. FILENAME: D:\DATA\FILENAME.WKS (*e.g.*, D041702A.WKS). **Make sure the filename you select will not overwrite data on the disk. The computer will not prompt you.**
 - i. LIST TYPE: FORMAT3
 - j. FILE TYPE: LOT
6. Click on EVALUATE (F7). This will begin the data transfer. Wait for the transfer to be complete before pressing ALT-T.
7. To transfer the run data you just transferred from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D041702A.WKS A: or COPY D041702*.*: A to copy all associated files). Press Ctrl and 1 (from the number keypad) to return to ISODAT.

Stanford University MAT252/Kiel III Carbonate Device Operation

V) Acid Drip Test Procedure

1. Click on CNF-B, SUPP-B, 1-CARBONATE UNIT. This will enter the Kiel support screen.
2. Open acid valve 10 and 20. Both can be open at the same time. Allow each to drop 2 to 3 drops of acid per reaction vessel or let them go until they time out (approximately 15 drops). Do not leave this unattended because it is possible for the vials to fill and flood valve system if counter fails to work correctly.
3. Close acid valve 10 and 20.
4. Click on TAKE MAG. This will remove both reaction vessels. Wait for both vessels to be removed before proceeding to Step 5.
5. Click on LOAD MAG. This will automatically load both reaction vessels.
6. Finished.



Figure P1: Finnigan Kiel III carbonate device oven and carousel assembly outside (upper left photo) and inside (upper right photo). In order to service the ports or to replace the carousel (middle left photo) it is necessary to remove vials from Port 1 (middle right photo) and 2 (bottom left photo). Once both vials are removed (bottom right photo) you can service ports.



Figure P2: Removing ice build up on the Finnigan Kiel III cold finger assembly. It is normal for ice to build up around the cold finger valve assembly (top left photo) but too much ice can cause problems and damage. Lower jack-stand to expose cold finger assembly (bottom left photo) and then remove LN₂ dewar. Use the blow dryer to expedite removal of ice around the top (top right photo) and around the bottom of the cold finger assembly (bottom right photo), do not pry or force the removal of the ice this could damage components of the entire assembly.



Figure P3: Removing and installing the sample carousel on the Finnigan Kiel III. To remove, carefully lift the carousel to free the shaft from the pivot base (left photo). Slide carousel out without touching the capillaries, counters, or pistons with the carousel or vials. To install, taking the same precautions as above, carefully slide the carousel in and gently place the carousel shaft onto the pivot base (middle photo). You will need to align the pin on the carousel shaft with the slot on the pivot base as well as the slot on the carousel top shroud with the vertical rod at the back of the unit (right photo).



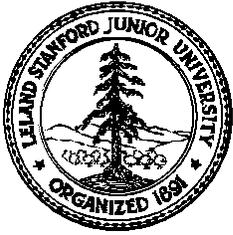
Figure P4: Clean the acid drip counters and capillaries on both Port 1 (left photo) and Port 2 (right photo) using 2-propanol and a cotton swab. Extra care should be taken not to alter the alignment between the counter and capillary.



Figure P5: Servicing the o-rings on the Finnigan Kiel III ports. Clean both the o-rings and stainless steel areas around Ports 1 (upper left photo) and 2 (upper right photo) using 2-propanol and a cotton swab. Place a small portion of Apiezon L on your index finger and lightly grease Ports 1 (lower left photo) and 2 (lower right photo).



Figure P6: Reloading the sample vials after cleaning and greasing Ports 1 and 2 (top left to bottom right).



Stanford University Stable Isotope Lab
 On-line Manual
 (Prepared by David A. Mucciarone)

Section 1C: Finnigan MAT252/24x Multiport Operation

I) Run terminated without error (NORMAL)

1. Click on ALT-T (upper left screen). This will exit CNF-A, ACON-A
2. Click on CNF-A, SUPP-A, and 2-MULTIPOINT CTRL. This will enter multiport support screen.
3. Note fore vacuum baseline (Fig. P1) and open the following valves in order. Click on valves 39, 13, 12, and 14 (Fig. 1). Allow vacuum to regain baseline.
4. Prepare and order sample tubes to be loaded into tube cracker manifold. Using a file or Dremel Tool to score the glass in the middle of the sample tube (Fig. P2). Make sure the scoring goes completely around the sample tube.
5. Click on CNF-A, EDIT-A, 3-SEQUENCE EDITOR Prepare a new table by copying a previous sequence. **The filename should be in the format operators first and last initial followed by the date (e.g., DM091702).** Enter new run information. Make sure that the reference refills (RR=120) are in the correct locations. When finished, press exit/save (F10) Sequence Editor, then Click on ALT-T.
6. Unloading/loading sample tubes in crackers (Fig. P3).
 - a. Open the bottom of the crackers by loosening the Swagelok cap fitting on the bottom. This can be done by hand or with two 9/16" wrenches.
 - b. Remove spent ampoules from crackers.
 - c. Place scored ampoules into cracker and secure with Swagelok cap fitting. Tighten fitting by hand or use two 9/16" wrenches. Be careful not to over tighten the fitting.
7. Click on CNF-A, SUPP-A, and 2-MULTIPOINT CTRL (Fig. 1).
 - a. Click on the Bank1 (or Bank2) on the upper left of the screen until the correct bank is selected.
 - b. Close valve 14.
 - c. Click on BANK (F7) and type BOPEN <ENTER>. This will open all 12 ports to the selected bank including valve B. The atmospheric pressure in the crackers will exit through valves B, 12, 13, 39.

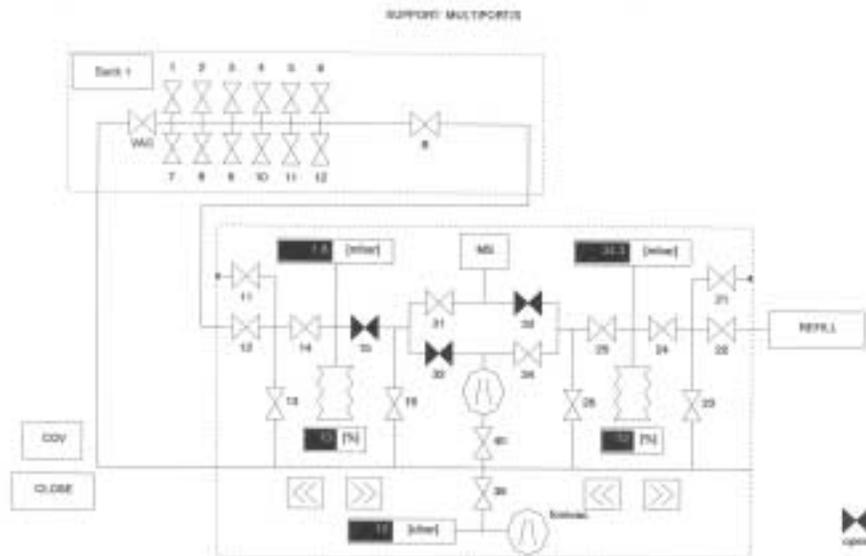


Figure1: Schematic representation of the MAT252 inlet system and multiport manifold.

8. Repeat Step 6a and 6c for the other bank.
9. Allow a minimum of 15 minutes for the crackers to evacuate before starting the run. This can be monitored from the inlet screen display on CNF-A, SUPP-A, and 2-MULTIPOINT CTRL.
10. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: (*e.g.*, DM091702)
 - b. START LINE: 1,
 - c. END LINE: 12, make sure start and end line numbers are correct (*e.g.*, 1 and 12 for a full bank; 1 and 24 for both banks)
 - d. MODE: DAY
 - e. FILENAME: **should be as follows: Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format operators first initial, date, followed by a letter beginning with A ending with .WKS (e.g., D091702A.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D091702A.WKS).
 - f. LIST TYPE: FORMAT1 (ONLY)
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: COL
10. Click SAVE (F9) to save sequence
11. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D091702A.WKS A:). Press Ctrl and 1 (from the number keypad) to return to ISODAT.
12. Click MEASURE (F5) to begin run. Make sure there is plenty of paper in printer to complete the run.
13. When the run display has refreshed (*i.e.*, updated screen display), you can crack all sample tubes (Fig. 4). Try and do this in an orderly manner so you do not miss any tubes.
14. It takes approximately 4 hours to run one bank of samples. Two complete runs (4 banks) can easily be run in a day. To accomplish this you will need to have your samples prepared in advance.



Stanford University Stable Isotope Laboratory Finnigan MAT252 mass spectrometer with dual inlet and 24 port manifold.

Stanford University MAT252/24x Multiport Operation

II) Run terminated with error

1. Click on ALT-T (upper left screen). This will exit CNF-A, ACON-A
2. Determine source of the problem and fix. When problem is resolved begin run where it was terminated.
3. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: reselect the correct table name (*e.g.*, DM091701).
 - b. START LINE: begin where the error took place.
 - c. END LINE: 24 if both banks are being run (12 if only 1 bank)
 - d. MODE: DAY
 - e. FILENAME: should be as follows: **Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format, operators first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D091702B.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D091702B.WKS).
 - e. D:\DATA\D091700B.WKS).
 - f. LIST TYPE: FORMAT1 (**ONLY**).
 - G. FILE TYPE: LOT
 - H. SAVE DATA TO DATABASE: COL
4. Click SAVE (F9) to save sequence. Make sure there is plenty of paper in printer to complete the run.
8. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad. Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D091702A.WKS A: or COPY D091702*.*: A to copy all associated files). Press Ctrl and 1 (from keypad) to return to ISODAT.
5. Click MEASURE (F5) to begin run.

Stanford University MAT252/24x Multiport Operation

III) Run interrupted by error

1) PART 1: Able to escape out or error

- A. If a gray window with a red bar will be displayed on the screen obstructing the normal display. Locate the source of the error (*e.g.*, high voltage out). Fix error then attempt to press ESC. If the error display disappears and the instrument appears to be working watch it carefully because it may actually be hung up and just going through the motions. It is best to just press ALT-T to terminate the run. If the error appeared a short time ago it is just a simple matter of restarting the sequence as follows
1. Click on ALT-T (upper left screen). This will exit CNF-A, ACON-A
 2. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10)
 - a. Table name: reselect the correct table name (*e.g.*, DM091701).
 - b. Start Line: begin where the error took place.
 - c. End Line: 24 if both banks are being run (12 if only 1 bank).
 - d. Mode: DAY
 - e. Filename: should be as follows: Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). **The filename should have the format, operators first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D091702B.WKS).** Type in the path and filename then press <Enter> (*e.g.*, the line should read as follows D:\DATA\D091702B.WKS).
 - f. List Type: FORMAT1 (**ONLY**)
 - g. File TYPE: LOT
 - h. Save DATA to database: COL
 3. Click SAVE (F9) to save sequence. Make sure there is plenty of paper in printer to complete run..
 4. Click MEASURE to begin run.
 5. If you were able to escape from the error or the run was able to continue the data was saved to the database (called ISOBASE) and to the filename you created. On the other hand, if you were not able to escape and had to refer to Part 2 of this section then the data was only saved to the database and not to the file you created in the SEQUENCE ACQ screen. This file will only have 128K. You will need to review the recover data section (IV) for instructions on how to obtain the run data.

Stanford University MAT252/24x Multiport Operation

2) PART 2: Unable to escape out or error

- A. If a gray window with a red bar will be displayed on the screen obstructing the normal display. If after looking for the fault and do not see one then attempt to press ESC. If the error display does not disappear to the point where the screen freezes then you will have to reboot the computer by following the reboot sequence.
1. Press Ctrl, Alt, Del to reboot computer.
 2. At the Load Real/32 prompt, Type N <Enter> this will load Win95
 3. At login prompt, Type sil <OK>
 4. Click on Scan Disk (on desktop). Let the program delete any fragments - DO NOT FIX THEM, just delete them.
 5. Restart the computer using the START button.
 6. At the Load Real/32 prompt, Type Y <Enter> this will load ISODAT v7.2 allow the microprocessor to reset before doing anything (message is displayed at top of screen).
- B. Continue as follows to prepare the run:
1. Click on ALT-T (upper left screen). This will exit CNF-A, ACON-A
 2. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10)
 - a. TABLE NAME: reselect the correct table name (e.g., DM091701).
 - b. START LINE: begin where the error took place.
 - c. END LINE: 24 if both banks are being run (12 if only 1 bank).
 - d. MODE: DAY.
 - e. FILENAME: should be as follows: **Path D:\DATA\filename.WKS (Filename is 8 characters.WKS). The filename should have the format, operators first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (e.g., D091702B.WKS).** Type in the path and filename then press <Enter> (e.g., the line should read as follows D:\DATA\D091702B.WKS).
 - f. List Type: FORMAT1 (**ONLY**)
 - g. FILE TYPE: LOT
 - h. SAVE DATA to database: COL
 3. Click SAVE (F9) to save sequence. Make sure there is plenty of paper in printer to complete the run.
 4. You need to remember that when a run is terminated by error in this way the data is saved to the database (called Isobase) only and not to the file you created in the SEQUENCE ACQ screen. This file will only have 128K. You will need to review the recover data section (IV) for instructions on how to obtain the run data.
 5. Click MEASURE to begin run.

Stanford University MAT252/24x Multiport Operation

IV) Recover run data from database

1. Click on CNF-A, EVAL-A, 1-OFF LINE
2. (6) SEQUENCE NAME: (*e.g.*, DM091701) Search by sequence name of run.
3. Click on DIRECTORY (Shift F5)
4. Click on TAG ALL (F5) if all of the runs need to be transferred to disk or use TAG (F6) to select the runs needed to be transferred.
5. Click on FORMAT (F8) and then enter the following exactly:
 - a. OUTLIER TEST: D2
 - b. STANDARD IDENT: SIL#2
 - c. LIST TYPE: NO
 - d. GRAPHICS: NO
 - e. ALPHA (CO3) CORRECTION: NO
 - f. ALPHA (H2O) CORRECTION: NO
 - g. ATOM% / APE REPORT: NO
 - h. FILENAME: D:\DATA\FILENAME.WKS (*e.g.*, D041700A.WKS). **Make sure the filename you select will not overwrite data on the disk. The computer will not prompt you.**
 - i. LIST TYPE: FORMAT1
 - j. FILE TYPE: LOT
6. Click on EVALUATE (F7). This will begin the data transfer. Wait for the transfer to be complete before pressing ALT-T.
7. To transfer the run data you just transferred from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D091702A.WKS A: or COPY D091702*.*: A to copy all associated files). Press Ctrl and 1 (from the number keypad) to return to ISODAT.



Figure P1: Perform a fore vacuum check by pressing the black FV Inlet button on the far right and monitor by reading the lower green bar (left photo). When finished press the black HV source button on the far right and monitor by reading the upper red bar (right photo).



Figure P2: Scoring sample tubes using file (left photo) or the Dremel Tool. With either technique it is necessary to make a score completely around the glass sample tube. Also, care should be taken not to break the glass sample tube or cutting your hand. With the file technique (left photo) apply enough pressure with your thumb to allow the file to cut into the glass while also allowing you to rotate the sample tube with the opposite hand. When using the Dremel Tool (right photo) set the Variac controller on 20 to 25 (out of 100), press the sample tube against grinding wheel and rotate making a score completely around the sample tube.

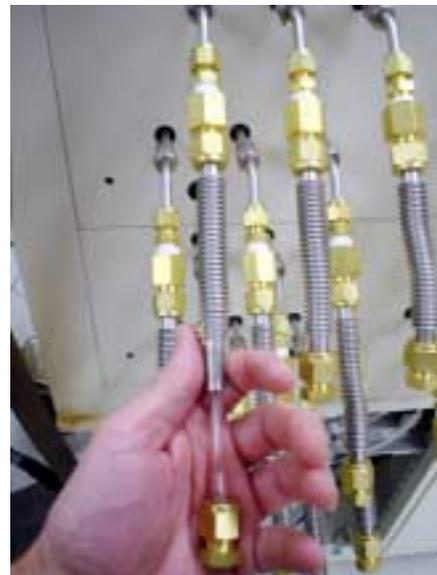
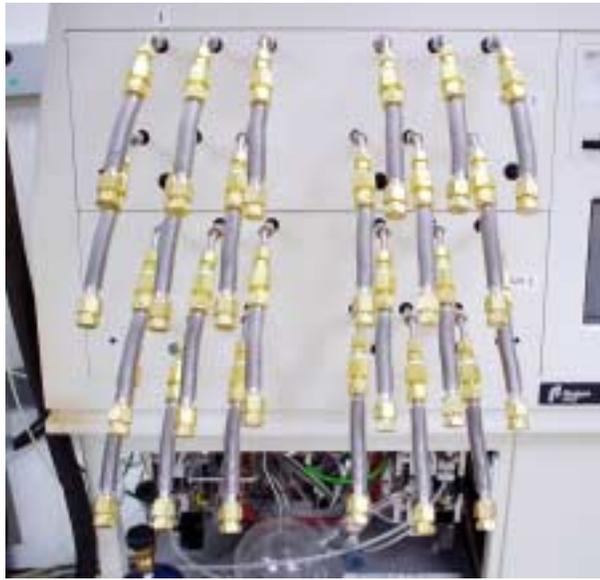


Figure P3: Loading sample tubes into tube crackers of the Finnigan MAT 252 24 port manifold (upper left photo). Note the orientation of the tube crackers on the 24 port manifold. Bank 2 (B2)-Tube cracker 1 (T1) is located at the upper left (top row) counting to the right to B2-T6, second row B2-T7 to B2-T12, third row Bank 1 (B1)-T1 to B1-T6, fourth (bottom row) B1-T7 to B1-T12. Select tube cracker (upper right photo) then loosen and remove end cap from tube cracker (lower left photo). Remove previous sample tube, if present, and

install new scored sample tube into tube cracker (lower right photo) then replace and tighten end cap.

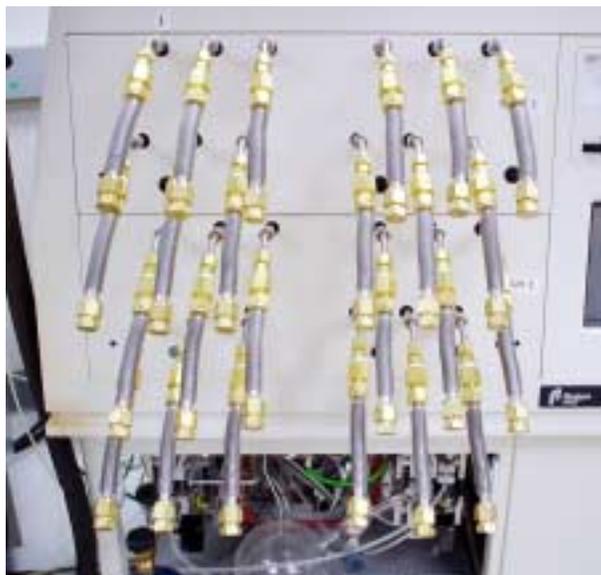
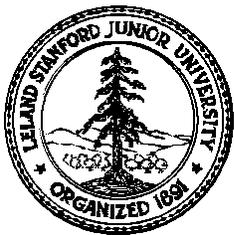


Figure P4: After you press measure (F5) you need to crack all sample tubes. It is best to do this in an orderly manner so you do not miss any samples. Crack sample tubes by bending the bellows tube until you hear the glass sample tube break (right photo).



Stanford University Stable Isotope Laboratory
On-line Manual
(Prepared by David A. Mucciarone)

Section 1D: Preparation of 100% Phosphoric Acid (H₃PO₄) for Kiel Carbonate Device

This procedure is designed to prepare approximately 425 ml of 100% phosphoric acid (H₃PO₄). The entire procedure should be performed under a fume hood. Goggles, rubber gloves and a lab coat/apron are required throughout the acid preparation.

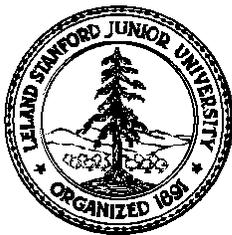
Materials: Ar grade 85% Phosphoric acid (H₃PO₄).

Equipment: Graduated cylinder 500 ml, 1000 ml beaker, hot plate or heating mantle with controller, ring stand with clamp, thermometer 200°C, storage bottle/flask with ground glass stopper.

1. Calibrate 1000 ml flask or beaker to account for 15% (~75 ml) loss of water during the heating process.
2. In a fume hood place 500 ml ACS grade 85% Phosphoric acid (H₃PO₄) into a calibrated 1000 ml beaker or round flask.
3. Insert thermometer and heat flask on hot plate or in heating mantle at 140°C for approximately 12 hrs or until enough water evaporates to reach the 15% calibration mark. Note: once temperature is stable at 140°C, remove thermometer.
4. When calibration mark is reached turn off hot plate or heating mantle and allow to cool.
5. Measure the density of the acid with a hydrometer. The specific gravity is 1.89 - 1.90. This is adequate for the Kiel. The acid resides in the oven at 70°C and flow well at this density. If the acid is more dense the acid begins to polymerize and clog the capillaries and tubing. The isotopic results are +/-0.03‰ for δ¹³C and +/- 0.05‰ for δ¹⁸O on standards

References:

- Bowen, R., 1966, Paleotemperature analysis: Elsevier Publishing Company, New York.
- Keish, B., J.W. Kennedy, and A.C. Wahl, 1958, The exchange of oxygen between phosphoric acid and water, *Anal. Chem.* v. 80, p. 4778-4782.
- McCrea, J.M., 1950, On the isotopic chemistry of carbonates and a paleotemperature scale: *Jour. Chem. Phys.*, v. 18, p. 849-857.
- Wachter, E.A. and J.M. Hayes, 1985, Exchange of oxygen isotopes in carbon dioxide-phosphoric acid systems: *Isot. Geosci.*, v. 52, p. 365-374.



Stanford University Stable Isotope Laboratory
On-line Manual
(Prepared by David A. Mucciarone)

Finnigan Delta_{plus} Carlo Erba NA 1500 Elemental Analyzer

Section 2A: Finnigan Delta_{plus} Operation Principles for elemental N, C, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ analyses

I) Operation Principles for $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ Analyses in organic and inorganic materials:

Before you can operate the Finnigan Delta plus mass spectrometer you must have training. Once trained the following procedures will serve as an operational guide. You should also read Section 2A on the ConFloII interface and Section 2C, which you can use as a quick reference guide to daily operation of the Delta plus/EA. The Delta plus mass spectrometer is connected to the Carlo Erba NA1500 elemental analyzer (Section 3) via a Finnigan ConFloII interface (Section 2A). The ConFloII interface allows us to connect two reference gases (N_2 and CO_2) and dilution gas (He) which is split off from the main EA carrier gas. Operation is as follows. Helium carrier gas constantly travels to the EA, ConFloII, and mass spectrometer. Samples in the zero blank carousel are combusted in an enriched oxygen environment in the combustion/oxidation column producing CO_2 , N_xO_x , SO_2 , and H_2O . The combustion/oxidation column, reduction column, and water trap work in series to insure that only N_2 and CO_2 enter the GC column and exit the EA through stainless steel tubing attached to Vent-M. See Section 3 for a detailed explanation on the removal and separation of gases in the EA. This tubing connects the EA to the ConFloII interface. At this point the gas enters an open split. Within the open split are four (4) fused silica capillaries, one for N_2 reference gas, one for CO_2 reference gas, one helium dilution of the sample coming from the EA and one connecting to the mass spectrometer. The reference gas and helium dilution capillaries are attached to individual solenoids; the mass spectrometer capillary is fixed. The fixed mass spectrometer fused silica capillary is connected to a SGE on/off valve mounted on top of the source housing (Fig. 1).

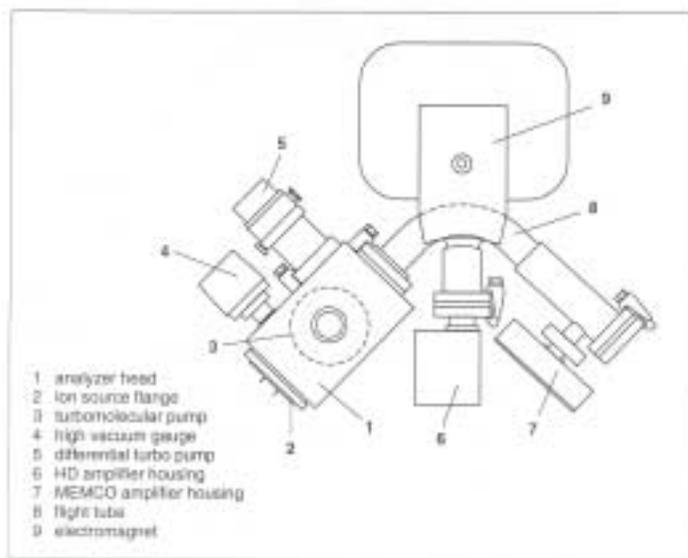


Figure 1: Schematic representation of the Delta plus analyzer system and the arrangement of components. Illustration is from Finnigan operations manual.

Once the gas passes through the SGE valves it enter the source through an inlet which simply directs the gas flow directly into the source block, more precisely the ionization chamber (see Fig 2). The gas flow in the ion source is always molecular. Attached to the source block are the filament (cathode), trap, ion extraction plates, and focusing plates (Fig. 3).

- The filament (cathode), often tungsten, emits electrons in a helical orbit under the influence of small source magnets in which the CO_2 gas molecules collide with the electron beam making a N_2^+ or CO_2^+ ion. The electron beam is at right angles to the ion trajectory.
- The trap collects all electrons not involved in the collision with the N_2 or CO_2 , and is positioned opposite the filament.
- The extraction plates accelerate ions out of the ionization chamber and direct the ions toward the exit slits and focusing plates. The accelerating voltage on the Delta plus is 3kV.
- There are a number of different focusing plates that are designed to progressively focus the ion beam leaving the source. The voltage is variable depending on the isotopic species being analyzed.
- The final focusing plate defines the maximum beam width prior to passage into the flight tube.

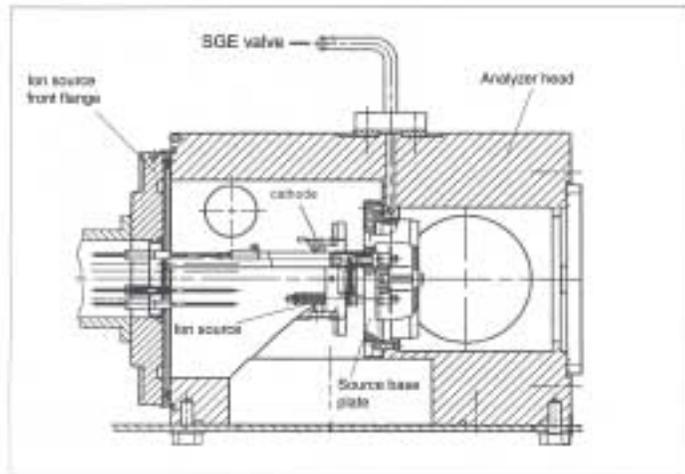


Figure 2: Schematic of Delta plus ion source within the source housing (analyzer head). Gases enter through the SGE valve into the ionization chamber. Illustration is from Finnigan users manual.

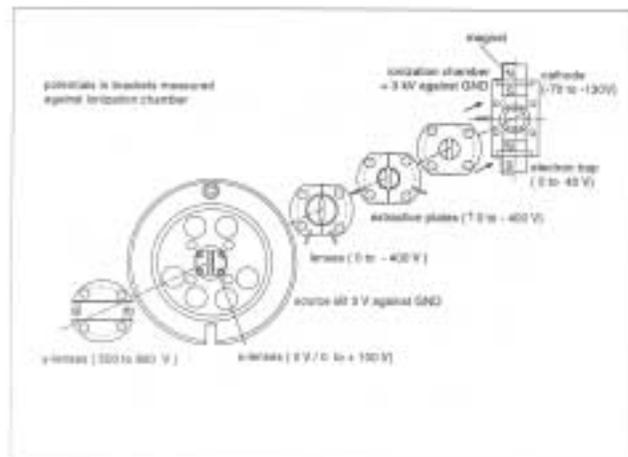


Figure 3: Schematic of Delta plus ion source components. Values are ion source potential in relation to ground at 3kV ion acceleration voltage. Illustration is from Finnigan user manual.

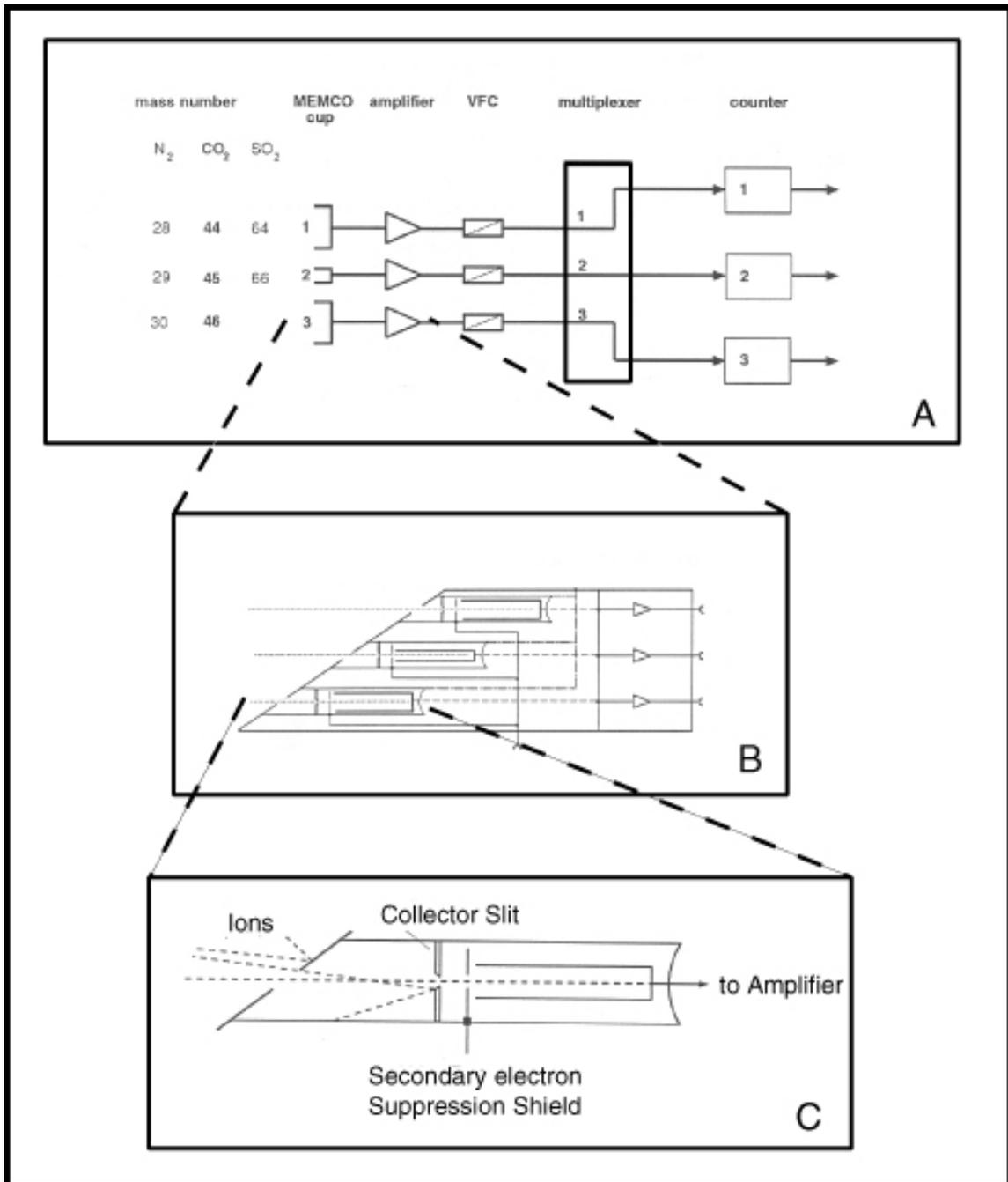


Figure 4: Schematic representation of faraday cup (collectors) on the Delta plus. A represents an overview of the collector system. B is an enlarged view of the collectors and amplifiers. Note that cup 2 is smaller than cups 1 and 3 but is more sensitive than cup 3 but less than cup 1. The resistor on cup 1 = 3×10^8 , cup 2 = 3×10^{10} , and cup 3 = 3×10^{11} for N₂ and CO₂. C is a detail representation of an individual collector.

The flight tube has a 90° deflection path (Fig. 1) having a radius of R fitted with a permanent magnet of strength B (BDAC). The Delta plus is equipped with an electromagnet with maximum field strength of 0.75 Tesla providing a mass range from 10 to 70 amu at full accelerating voltage of 3kV. Therefore the radius is fixed and the magnet is variable but constant for any given gas species. At a constant accelerating voltage (V) and magnet strength, the lighter mass will be deflected (bend) more than

the heavier mass. In the case of CO₂, mass 44 is bent more than 45 and 46. However, in order to analyze another gas such as N₂ or SO₂ with a fixed magnet, the accelerating voltage needs to increase or decrease, respectively or the accelerating voltage can remain the same and the magnetic field strength can be varied. Therefore, the relation of all these parameters is given as:

$$m/z = K * H^2$$

Where: m/z is the mass to charge ratio
 K = R²/2U = constant
 H is the magnet strength
 R is the Radius of flight tube
 U is the acceleration voltage

After leaving the flight tube, the separate ion beams of different masses are directed into 3 (1 major and 2 minor) collectors (Faraday cups) depending on the type of mass spectrometer (Fig. 4). These cups are grounded through high ohm resistors. As the ion current passes to ground the drop in the resistor acts as a measure of the ion current. In addition, each collector also has a feedback resistor, therefore each amplifier can be matched to the isotope collected in a particular faraday cup. There are two types of collectors, major and minor. The major collector is usually larger and less sensitive than the minor collector. These collectors can either be fixed to a correct spacing for a particular set of masses or adjustable. In the case of N₂ or CO₂ a triple collector mass spectrometer can detect all three masses (28-major, 29-minor or 44-major, 45-minor, and 46-minor) and calculate δ¹⁵N and δ¹³C in one run without changing feedback resistors. The ISODAT software cup configuration utility does this automatically.

The signals received by the collectors are amplified and transferred to voltage to frequency converters (VFC) on a system control board in a microprocessor. This system board basically digitizes the analog signal for processing by an interfaced computer. The analog and digital output is then sent to disk and a printer. In the course of one analysis for δ¹⁵N and δ¹³C on the Delta plus mass spectrometer, one reference and one sample injection are measured to determine the concentration and isotopic composition. All of the data are expressed in conventional delta (δ) notation, where the isotopic ratio of δ¹⁵N/δ¹⁴N is expressed relative to Air and δ¹³C/δ¹²C relative to the international PDB standard (Eq. 1) as defined by:

$$\delta \text{ ‰} = [(Ratio_{\text{sample}} - Ratio_{\text{standard}})/(Ratio_{\text{standard}})] \times 1000 \quad (\text{Eq. 1})$$

PDB refers to a Cretaceous belemnite called *Belemnitella americana* from the Peedee formation in South Carolina and is defined as 0 for both δ¹³C and has been exhausted (however, there may be a few labs that still have a small quantity remaining). The carbon standard NBS-21, USGS-24 (both graphite), NBS-22 (oil), and NIST-1547 (peach leaves), where NBS is an acronym for the National Bureau of Standards which was replaced by NIST or the National Institute of Standards and Technology, and USGS or U.S. Geologic Survey are standards used to calibrate to PDB. The CO₂ reference gas used is research grade and has been calibrated to PDB using these standards. Nitrogen isotopes are compared to Air, which is defined as zero. The N₂ reference gas we use is research grade and has been calibrated to Air using IAEA-N1 and IAEA-N3, USGS-26, where IAEA is an acronym for the International Atomic Energy Agency. To reduce the possibility of exhausting these international standards, laboratories establish working standards that have been calibrated against various known standards and with other laboratories, to use routinely in the laboratory. Listed in Table 1 are the isotopic compositions of commonly used standards, most of these can be obtained from the National Institute of Standards and Technology (NIST) in Gaithersburg, MD 20899-0001. For more information consult the reference list below.

Now that you have a general understanding on how the Finnigan Delta plus operates you can continue with Part B of this section to see how the gases generated by the EA move through the system and how they are analyzed. Consult Section 2A for a discussion on the ConFloII interface and Section 2 C for the quick reference guide to the operation of the EA and the Delta plus.

Table 1: Isotopic compositions of standard reference materials (Coplen et al., 2002).

Standard.	Description (‰)	$\delta^{15}\text{N}_{(\text{Air})}$ (‰)	$\delta^{18}\text{O}_{(\text{SMOW})}$ (‰)	$\delta^{13}\text{C}_{(\text{PDB})}$ (‰)
PDB	Peedee belemnite		30.91	0.00
SMOW	Standard Mean Ocean Water		0.00	
Air	Air	0.00		
IAEA-N1	Ammonium sulfate	0.43		
IAEA-N3	Potassium nitrate	4.69		
USGS-26	Ammonium sulfate	53.62		
NBS-19	TS limestone		28.65	1.95
USGS-24	Graphite			-15.99
NBS-21	Graphite			-28.10
NBS-22	Oil			-29.74

II) Chromatography and Isotopic Analysis

The Delta plus is connected to an EA via a ConFloII open split interface. Helium carrier gas flowing through the entire system in conjunction with the length of the GC column and the temperature of the GC oven will control the retention times of the N_2 and CO_2 . These parameters have been selected so that we can maximize both separation and chromatography (shape) of the sample peaks. These two factors are important for a couple of reasons. First, separation between peaks is needed so that the Delta plus mass spectrometer can peak jump from N to C. Peak jumping involves changing the electromagnet current (BDAC) while maintaining the source voltage (HVDAC) constant. This process takes time and therefore adequate time must be budgeted to allow completion of this task. Second, proper peak shape is needed to obtain accurate concentration and isotopic measurements. Long tails on peaks will increase error in both calculations. Our system is setup to perform a slow jump routine between the nitrogen and carbon run. Our premise for using this routine is because it allows more maximum tuning of the source prior to analyses of N and C. The fast peak jump routine requires the software to be instructed on how to change the electromagnet current. This value will not be adjusted during the entire run sequence. All things remaining equal this routine will perform satisfactory, however, through the course of a given run sequence the electromagnet and/or source voltage can drift. Thereby introducing the possibility of errors and instability. If the retention times between peaks are close due to EA limitations or because of preference then the operator will benefit by using the fast peak jump routine. In reality, the mass spec will drift slightly over the course of a run sequence. So, by modifying the configuration and parameters of the EA we have created enough separation between the N and C peaks in a manner that we feel maximizes concentration and isotopic precision without compromising chromatography. See Table 1 for the configurations of the Delta plus/EA.

A typical run for nitrogen and carbon on the Delta plus is as follows. The run begins with a peak jump of the electromagnet current (BDAC) to N_2 and centers the high voltage (HVDAC) on mass 29 or beam 2. When complete a new graphics display will appear on the screen. A signal is sent to the EA to begin 1 second into the run and turn off at 25 seconds. The ConFloII solenoid will activate at approximately 14 seconds into the run indicated by the start of the LCD timer and the activation of the oxygen injection solenoid on the EA. When the EA timer reaches 20 seconds, the zero blank carousel will rotate one position dropping the sample into the combustion/oxidation column. At 90 seconds into the run a signal is sent to the ConFloII interface and N_2 reference gas is injected into the mass spectrometer for 20 seconds. The timer on the EA will reset at 120 seconds. At about 175 seconds into the run the N_2 peak from the sample will elute out of the 4 m GC column. Depending on the amount of N in the sample the peak will have a retention time of approximately 50 to 80 seconds. One second prior to the end of the nitrogen run the helium dilution solenoid is activated and at 275 seconds the nitrogen portion of the run is terminated (Fig. 1).

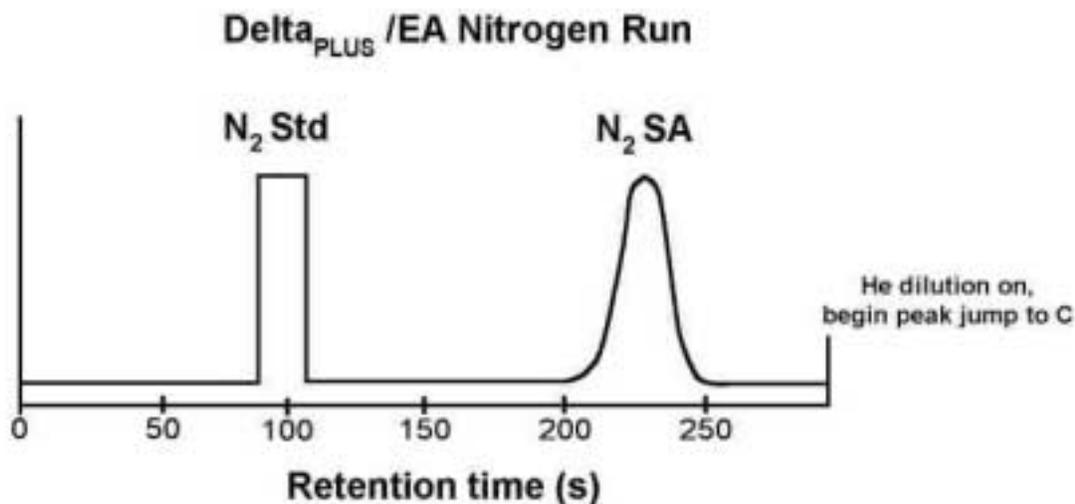


Figure 1: Schematic representation of the nitrogen portion of a nitrogen/carbon analysis. Notice that the N₂ reference gas is injected prior to the unknown N₂ sample peak.

A slow peak jump routine is performed as the instrument prepares to analyze CO₂. This routine requires about 75 seconds and involves a change in electromagnet current (BDAC), injection of CO₂ reference gas from the ConFloII interface, and centers the high voltage (HVDAC) on mass 45 or beam 2. When complete a new graphics display window will appear. Approximately 18 seconds into the run the CO₂ peak from the sample will elute out of the 4 m GC column. Depending on the amount of C in the sample the peak will have a retention time of approximately 75 to 130 seconds. At 185 seconds a signal is sent to the ConFloII interface and CO₂ reference gas is injected into the mass spectrometer for 20 seconds. One second before the end of the run the helium dilution is turned off and the carbon portion of the run has ended and the analysis of the sample is completed (Fig. 2). This method of analysis continues until the run sequence is finished.

The above discussion has been about obtaining elemental and isotopic values from N and C from the same sample. Table 2 is a quick reference on how the EA and ConFloII are configured to run N and C on the same sample. It is also possible to run only N or C where peak jumping is not required. It is however, necessary to configure a Method in ISODAT to perform this task correctly. In addition, it is also possible to configure the EA differently to run O in the form of CO (Section 2D) or S in the form of SO₂ (Section 2E). To do this it is necessary to reconfigure the EA with different reactor and/or reactor/reduction columns and GC columns. New Methods will need to be created in ISODAT to accommodate the modifications in the EA. Aside from running an EA on our Delta plus, other peripherals can be attached that run via continuous flow. For example we have a HP5890 GC configured to do compound specific GC-C-IRMS (Section 2F).

It is necessary to review and understand the operation of the elemental analyzer before operating the mass spectrometer. This section was written as a synopsis and as a guide to the safe operation of the Delta plus. You will receive all of the necessary information for operating the instrument from your training. However, you can find more details on the mass spectrometer and elemental analyzer in the sorted Finnigan Delta plus and Carlo Erba user manuals.

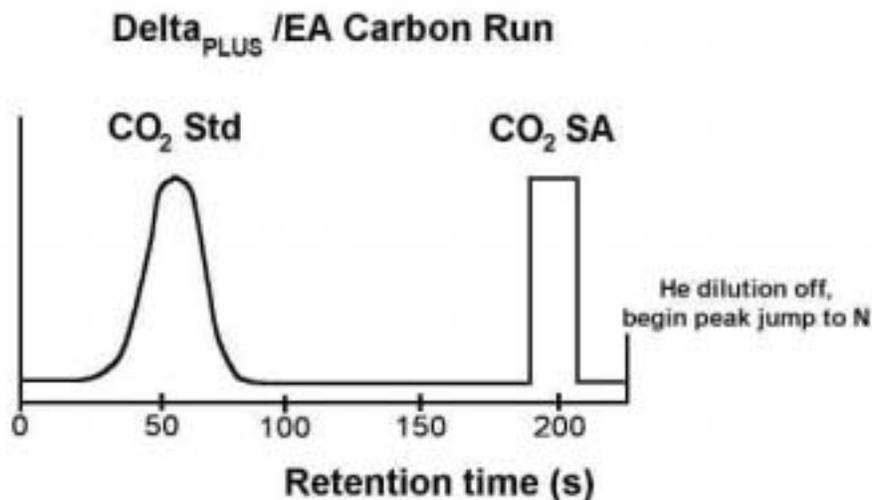


Figure 2: Schematic representation of the carbon portion of the nitrogen/carbon analysis. Notice that unlike the nitrogen run the CO₂ reference gas is injected after the unknown CO₂ sample peak this is because of the slow peak jump routine from N to C.

Table 2: Set up parameters for C and N analysis using the Carlo Erba Na1500 Series 2 elemental analyzer.

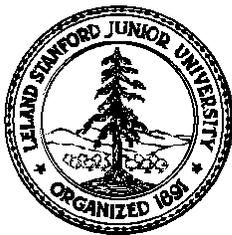
Instrument Temperatures (°C)	
Combustion oven	1020
Reduction oven	650
Chromatographic column	60
Filament	190
Flow Rates (ml min⁻¹) and gas pressures	
Helium carrier gas, Grade 4.6, Zero (90 - 100kPa on EA)	55-65
Oxygen Grade 4.3, UHP (100 - 105kPa on EA)	25-30
Nitrogen Research Grade 6.0 (100 - 105kPa on CFII)	
Carbon dioxide Research Grade 4.6 (100 - 105kPa on CFII)	
Helium Grade 4.6, Zero (90 - 100kPa on CFII)	
Element Retention Times (seconds)	
Nitrogen as N ₂ reference gas injection	80-100
Nitrogen as N ₂	175-260
Carbon as CO ₂	20-120
Carbon as CO ₂ reference gas injection	185-205
Additional Parameters on EA (seconds)	
Cycle time	110
Sample time begin/end	20 & 21
Oxygen injection	70
Run Parameters (seconds)	
Run time for N	265
Peak jump to C	~90-100

Table 2 (cont.): Set up parameters for C and N analysis using the Carlo Erba Na1500 Series 2 elemental analyzer.

Run time for C	225
Peak jump to N at end of run	30
Run time for N and C	630s (10.5 min)
Column Configuration (cm)	
Quartz combustion column (1 x w)	45.0 x 1.8
From bottom to top:	
-Quartz wool	4.0
-Silvered cobaltic oxide (Co ₃ O ₄ + Ag)	6.0
-Quartz wool	0.5
-Chromium oxide (Cr ₂ O ₃)	10.5
-Quartz wool	0.5
Quartz reduction column (1 x w)	45.0 x 1.8
From bottom to top:	
-Quartz wool	4.0
-Reduced copper wires (approx. 150g)	30.0
-Quartz (silica) chips	9.0
-Quartz wool	1.5

III) References:

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Stanford University Stable Isotope Lab
On-line Manual
(Prepared by David A. Mucciarone)

Section 2B: Finnigan Delta_{plus} and Carlo Erba NA 1500 Elemental Analyzer with ConFloII interface

I) Operation Principles for elemental N, C, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ Analyses:

Before you can operate the Finnigan Delta plus mass spectrometer you must have training. You should also consult Section 3 of this on-line manual (Carlo Erba NA1500 Series 2 elemental analyzer - Principles of Operation). The objective of this section is to give you an understanding on how the Finnigan ConFloII interface operates using the Finnigan Delta plus and Carlo Erba NA1500 as an example. Most of the information outlined in this section can be found in the Finnigan operations manual, however, it is synthesized here so that it applies to the instrumentation we have in the Stanford Stable Isotope Laboratory. Once trained the following procedures will serve as an operational or reference guide. In our laboratory the Delta plus mass spectrometer is connected to the Carlo Erba NA1500 elemental analyzer (EA) via a Finnigan ConFloII interface. The ConFloII interface allows us to connect two reference gases (N_2 and CO_2) and dilution gas (He) which is split off from the main EA carrier gas. Operation is as follows. Helium carrier gas travels to both the EA and the ConFloII into the Delta plus mass spectrometer. The helium carrier gas travels through the EA (~60 to 65 ml/min) carousel, combustion and reduction columns, water trap, 4m GC column and exits the instrument through the 1/8" (3.175mm) stainless steel tubing attached to Vent-M (CE NA1500). This tubing connects the EA to the ConFloII interface. At this point the gas enters an open split (Fig. 1). The mass spectrometer requires molecular flow ($<10^{-4}$ Torr) in order to ionize gas efficiently. This is accomplished in the open split. Gases produced in the EA are transported by helium carrier gas flowing at 60 to 65 ml/min and enter the open split. Since the mass spectrometer capillary is so small (0.1mm ID) only a small portion ($<10\%$) of sample and carrier gas can enter the mass spectrometer. The essentially reduces the flow to ~0.3 ml/min and allows the ion source to operate at a 10^{-6} Torr vacuum.

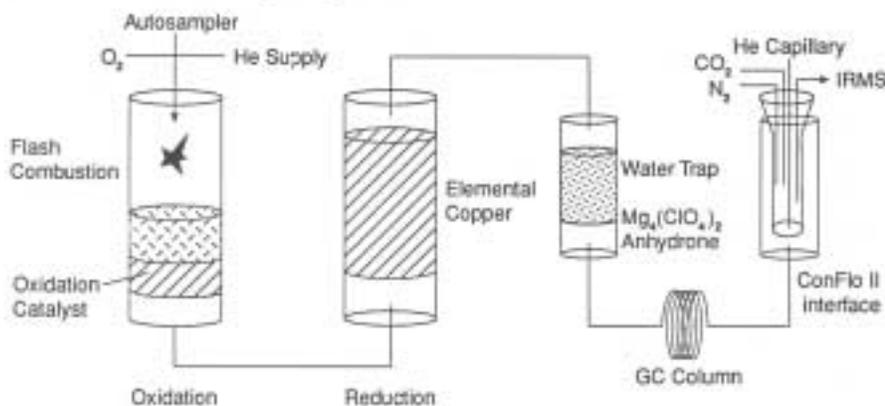


Figure 1: Schematic representation of the Carlo Erba NA1500 setup for Nitrogen and Carbon connected to the Finnigan ConFloII interface. IRMS refers to isotope ratio mass spectrometer in our laboratory it is a Finnigan Delta plus.

Within the open split are four (4) fused silica capillaries, one for N_2 reference gas 0.025mm ID x 0.3mm OD), one for CO_2 reference gas (0.025mm ID x 0.3mm OD), one for helium dilution (0.18mm ID x

0.27mm OD) of the sample coming from the EA and one connecting to the mass spectrometer (0.10mm ID x 0.3mm OD). The reference gas and helium dilution capillaries are attached to individual solenoids, which allows for gas injection control. The mass spectrometer capillary is fixed and is set below the non-injected reference and helium dilution capillaries. Without any injection of reference gas or helium dilution the flow of gas from the EA through the open split would be as illustrated in Figure 2. You will only find the ConFloII system in this position when gas(es) from the EA do not require helium dilution which is often the case when running nitrogen or when running small samples for carbon without analyzing for nitrogen. In most natural abundance samples such as soils, sediment, and plants it is common that the amount of carbon present exceeds nitrogen (C:N) anywhere from 5:1 to 15:1. Therefore most samples when analyzing

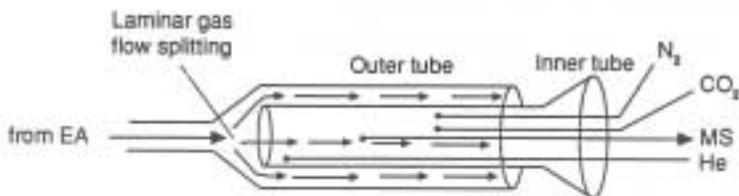


Figure 2: Schematic of the Finnigan ConFloII interface. Gas from the elemental analyzer (EA) enters the open slit where over 90% of the gas will exit the slit and the remaining gas enters the mass spectrometer (MS) capillary. Reference gas from the N₂ and CO₂ capillaries is always flowing and vents the open slit by the helium carrier flow traveling through the apparatus. Note that when the reference capillaries are not being injected they are above the MS capillary therefore, they do not enter the MS capillary. The helium dilution capillary is in the injection position, it is normally located above the MS capillary unless injection is required.

for N and C from the same sample run require helium dilution of the CO₂ in order to reduce the amount of gas so that the sample can be analyzed by the Delta plus. So depending on the C:N ratio of the samples run on the EA, helium dilution may be required and the gas would behave as illustrated in Figure 3. Depending on how much helium dilution is required there is a regulator and pressure gauge on the front panel of the ConFloII housing that allows the user to preset a pressure for a run sequence. When using helium dilution on any gas(es) the user must keep in mind that changing helium dilution pressure will affect the isotopic composition of the gas and the amount of gas entering the mass spectrometer. The user should

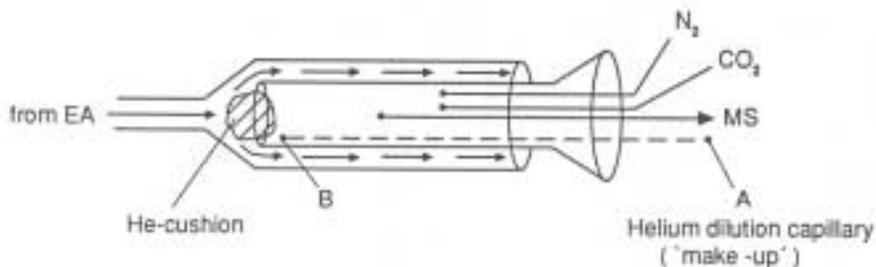


Figure 3: Schematic of the Finnigan ConFloII interface injecting helium. Often when N and C are analyzed from the same sample helium dilution is required. As with the two reference capillaries the helium in the dilution capillary is always flowing. When required the helium dilution capillary moves from position A (not injecting or off) to B (injecting or on). Depending on the preset pressure of the helium dilution regulator, less gas from the EA is allowed to enter the inner portion of the open split reducing the amount of sample gas entering the mass spectrometer (MS) capillary.

predetermine the amount of helium dilution required for any given run sequence, set the gauge pressure using the regulator, and do not change the pressure until the beginning of another run sequence. A run sequence consists of three conditioning standards, two capsule blanks, suite of standards for determining concentrations and isotopic composition, a suite of unknowns, ending with a suite of standards. This run sequence can range in length depending on a number of different factors such as sample size, type, etc. Since the injection of the helium dilution is consistent at a given pressure once set and standardized it is easy to account for this affect.

To standardize the unknown samples or standards a reference gas, either N_2 or CO_2 , is introduced via the ConFloII interface (Fig. 4). Like the helium dilution the pressure can be predetermined prior to a run sequence to be in the middle of the expected average unknown and standard range. However, unlike the helium dilution the isotopic composition will not change if the pressure is adjusted in between individual runs in a sequence. You also need to keep in mind that the reference gases are always flowing. When they are not in the injection position the gas(es) are simply venting out of the open slit (position A in Fig. 4). When the capillary is in the injection position (position B in Fig. 4), as with the gas(es) coming from the EA, only a small portion of the reference gas enters the fixed mass spectrometer (MS) capillary the rest vents out of the open split. In most cases an adjustment of these pressures is not necessary or encouraged because this is a means of monitoring any change in the sensitivity of the mass spectrometer ion source. The pressures on the gauge are adjusted so that the voltage intensity on beam 2 on the mass spectrometer is set to approximately 2 volts. This works well for most run sequences. Before making adjustment to reference gas pressures consult with the lab manager.

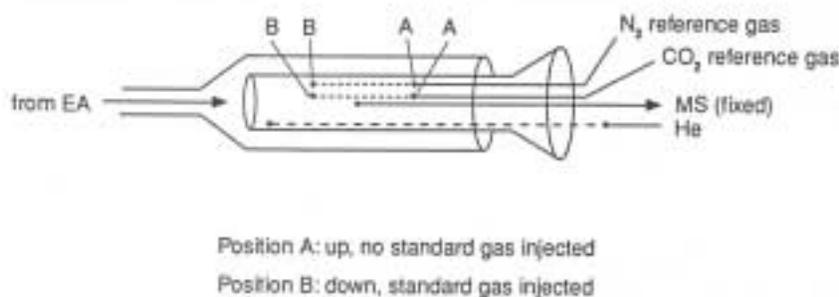


Figure 4: Schematic of the Finnigan ConFloII interface injecting N_2 and CO_2 reference gases. Position A and B represents the position of the reference gas capillaries without or with gas injection, respectively. Remember the reference gases from each of these capillaries are always flowing. The position of the capillary determines whether or not gas is introduced into the fixed mass spectrometer (MS) capillary.

Now that we have an understanding on how the ConFloII operates let's take a look at an individual sample run and the position of each capillary in the open split (Fig. 5). For example, with the EA setup to run carbon and nitrogen the following procedure happens:

1. Instrument is in Nitrogen Configuration a peak center is performed prior to starting each run.
2. The EA is started and a sample is combusted (A in Fig. 5)
3. While the sample is combusting and being filtered and separated in the GC column nitrogen reference gas is injected for 20 seconds. (B in Fig. 5)
4. Nitrogen from the unknown/standard elutes from the GC column and enters the mass spectrometer ion source and is later detected by the faraday cups.
5. The instrument software (ISODAT) then peak jumps the electromagnet on the Delta plus mass spectrometer to Carbon Configuration to detect the carbon dioxide from the unknown/standard in the same manner as nitrogen (X in Fig. 5). A peak center is performed during the peak jump process.

6. Helium injection begins and continues until end of run (C in Fig. 5).
7. Carbon dioxide from the unknown/standard elutes from the GC column and enters the mass spectrometer ion source and is later detected by the faraday cups.
8. After CO₂ from the unknown/standard is complete CO₂ reference gas is injected for 20 seconds. (D in Fig. 5)
9. Instrument software (ISODAT) peak jumps back to the Nitrogen Configuration, a peak center is performed and the next sample in the sequence is run.

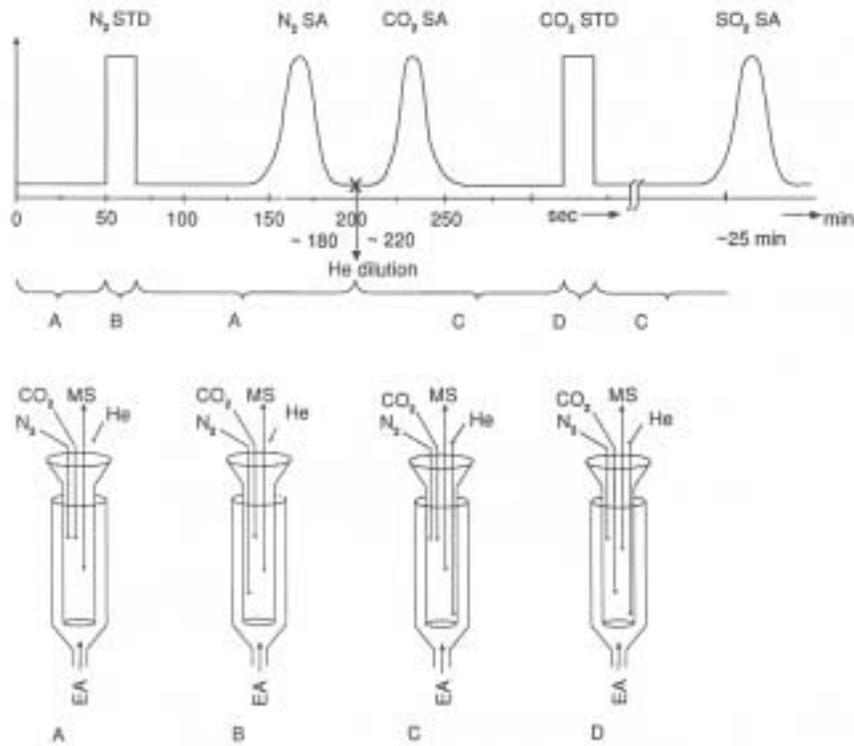
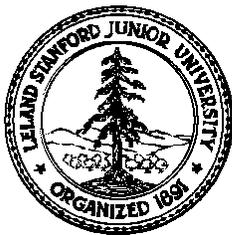


Figure 5: Schematic of a C and N run on the Finnigan Delta plus/EA illustrating the various configurations of the ConFloII interface. A denotes no injections, B and D represents N₂ and CO₂ reference gases, respectively, and C is for helium dilution. The X represents the point when the instrument software peak jumps from N to C.

It is necessary to review and understand the operation of the elemental analyzer and the mass spectrometer prior to operating these instruments. This section was written as a guide that will help you better understand continuous flow mass spectrometry and how it relates to the instruments in the Stanford Stable Isotope Laboratory. You can find more details on the mass spectrometer, elemental analyzer, and the ConFloII interface in the sorted Finnigan Delta plus and Carlo Erba manuals.



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Section 2C: Finnigan Delta_{plus}/Carlo Erba Elemental Analyzer Operation

I) Run terminated without error (NORMAL)

1. Click on ALT-T (upper left screen). This will exit CNF-A, ACON-A.
2. Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M to Vent-R (Fig. P1).
3. Remove quartz insert from EA reactor column:
 - a. Loosen purge valve on top of carousel (Fig. P2).
 - b. Next open the purge valve on top of the carousel then loosen the fittings connecting the carousel to the reactor column and helium input line. Move carousel to the side so the insert can be removed without obstruction (Fig. P2).
 - c. Carefully remove insert from reactor. Care should be taken when handling the insert because it is at 1020°C. Place hot insert into waste beaker (Fig. P3).
4. Install new quartz insert into EA reactor column:
 - a. Put approximately 7mm of quartz wool into the bottom of the insert (Fig. P3).
 - b. Carefully slide the quartz insert into reactor column until it stops (do not push or force insert, Fig. 3). The insert should stop approximately 5mm above top of reactor column). If the insert falls below the top of the reactor column add an appropriate amount of quartz wool to the reactor column (not the insert) then slide the insert in again.
 - c. With the insert in the proper position, gently push the insert down until it is flush with the top of the reactor column (do not force).
 - d. Reposition the carousel onto the reactor column and secure both connections at the reactor column and the helium carrier line (Fig. P4).
5. Carousel Operation (Fig. P5):
 - a. Front panel: Toggle switch up = 32 hole sample drum
Toggle switch down = 50 hole sample drum
Black push button = advances drum forward 1 position
Round switch up = on (red light on)
Round switch down = off (red light off)
 - b. Rear panel: Switch on the rear panel is to align the carousel after swapping out sample drums.
Toggle switch up = advance sample drum by steps
Toggle switch down = reverse sample drum by steps
6. Load samples into carousel (Fig. P6):
 - a. Loosen the 3 thumbscrews on the top of the carousel (loosen back two first then front) and fold out of the way. Flip back the carousel lid to expose sample drum.
 - b. Using forceps carefully load samples into sample drum. If first sample is not in hole 1, record start position on sample log sheet. This will be helpful if there is a problem with the run (e.g., sample capsule wedges in hole and does not drop, samples need to be removed).
 - c. Once samples are loaded, close lid and secure using the 3 thumbscrews. Tighten back two first then front.
 - d. Allow carousel to purge for 2 minutes before closing purge valve on the top of carousel lid.
7. Check magnesium perchlorate water trap. Change when the upper 10 to 15mm of magnesium perchlorate granules appear fused together (Fig. P7).
 - a. To change loosen red caps on both ends of trap and remove. Teflon/silicon seals usually stick to the glass trap, remove seals, check for cracks and replace if necessary otherwise clean and use.
 - b. Place 5 to 7mm of quartz wool in one end of a clean trap (located in oven) and fill with magnesium perchlorate granules and gently tap trap on bench top to compact granules. Be sure to leave approximately 5 to 7mm of space in order to insert quartz wool into the open end.

- c. Clean both faces of trap and reassemble. Place seals in red caps with Teflon portion of seal against the metal flange and secure finger tight.
- d. Magnesium perchlorate from used trap needs to be removed and placed into the waste container. Glass trap can then be cleaned with water and placed into the oven to dry.
8. Perform a leak check EA (Fig. P8).
 - a. Place a plug over Vent-M, release pressure on M helium regulator on EA (turn black/silver knob counterclockwise).
 - b. If there is a leak the pressure on the M helium gauge will decrease. If this occurs check for leaks. The most common source of leaks will be at the carousel connection to the reactor column, seals in water trap, or the carousel lid seal. Make sure to restore helium pressure to 90kPa before checking for leaks. Repeat Steps 8a and 8b if necessary until leak is repaired.
 - c. If there is no leak, remove plug on Vent-M and restore helium pressure to 90kPa.
9. Select the helium dilution on ConFloII interface box (top black knob). Record helium dilution along with N₂ and CO₂ reference gas gauge pressures on sample log sheet (Fig. P9).
10. Check tuning on Delta_{plus} source and perform peak shape on CO₂.
 - a. Click on CNF-A, SUPP-A, and 1-CONFLO II CTRL. This will allow control of ConFloII valves.
 - b. Click on REF1 ON (F6) or click on the REF 1 hourglass icon. This will introduce CO₂ into the mass spectrometer via the ConFloII interface (Fig. 1).
 - c. Click on CNF-D, ACON-D, and 1-INSTR. CONTROL.
 - d. Click on CUP CFG (Shift F10), left click on CO2-CONF then right click or <ENTER> to select. This will select the correct magnet and source voltage setting for CO₂.
 - e. Click on CENTER (F6) this will center the source voltage, when finished click on SHAPE (F8) followed by MEASURE (F5) to perform a peak shape on mass 44, 45, 46. When complete click on EXIT (F10). Now perform this same procedure on N₂.
11. Check tuning on Delta_{plus} source and perform peak shape on N₂.
 - a. Click on CNF-A, SUPP-A, and 1-CONFLO II CTRL. This will allow control of ConFloII valves.
 - b. Click on REF1 OFF (Shift F6) or click REF 1 hourglass icon then click on REF2 ON (F8) or click on the REF 2 hourglass icon (Fig. 1). This will stop CO₂ from entering the mass spectrometer and introduce N₂ into the mass spectrometer via the ConFloII interface. Click on ALT-T.
 - c. Click on CNF-D, ACON-D, and 1-INSTR. CONTROL. This will allow control of Delta_{plus} source.
 - d. Click on CUP CFG (Shift F10), left click on N2-CONF then right click or <ENTER> to select. This will select the correct magnet and source voltage setting for N₂.
 - e. Click on CENTER (F6) this will center the source voltage, when finished click on SHAPE (F8) followed by MEASURE (F5) to perform a peak shape on mass 28, 29, 30. When complete click on EXIT (F10).

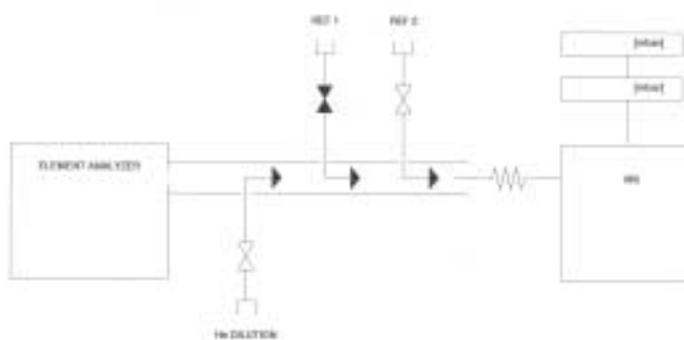


Figure 1: ConFloII reference gas and helium dilution control (CNF-A, SUPP-A, and 1-CONFLO II CTRL) screen. Black hourglass indicates open valve, white represents a closed valve.

12. Click on ALT-T to exit CNF-D, ACON-D, and 1-INSTR. CONTROL.
13. Click on ALT-T to exit CNF-A, SUPP-A, and 1-CONFLO II CTRL.
14. Click on CNF-A, EDIT-A, and 2-SEQUENCE EDITOR. Prepare a new table, which can be done by copying a previous sequence, and modify as needed. The filename should be named after the type of

samples being loaded (e.g., ODP1098-1, ODP1098-2, etc.). Enter new run information. Make sure you have selected the correct METHOD when creating a sequence table. When finished, save and exit (F10) Sequence Editor, then Click on ALT-T.

15. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: (e.g., ODP1098-1)
 - b. START LINE: 1
 - c. END LINE: 31 (make sure start and end line numbers are correct).
 - d. MODE: NIGHT
 - e. FILENAME: should be as follows: Operators first initial, date, followed by a letter beginning with A followed by .WKS (e.g., D091701A.WKS). The line should read as follows D:\DATA\D091701A.WKS).
 - f. LIST TYPE should be FORMAT3
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: NO
16. Click SAVE to save sequence.
17. When the base level is between 660 to 725 μ V and stable (Fig. P10), switch the stainless steel line connection EA to the ConFloII interface from Vent-R to Vent-M (Fig P1). The baseline level will drop about 50-70 μ V and stabilize. This is normal because you are pulling a vacuum on the mass spectrometer fused silica capillary.
16. Click MEASURE to begin run. Make sure there is plenty of paper in printer to complete the run.
18. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (e.g., at prompt D:\DATA> Type COPY D091701A.WKS A:). Press Ctrl and 1 (from the number keypad) to return to ISODAT.



Stanford University Stable Isotope Laboratory Finnigan MAT Delta_{plus} mass spectrometer, Carlo Erba NA1500 Series II elemental analyzer, Costech Analytical zero blank carousel, and Finnigan MAT ConFloII open split interface.

Stanford University Delta_{plus}/Carlo Erba Elemental Analyzer Operation

II) Run terminated because of problem

1. Click on ALT-T (upper left screen). This will exit CNF-A, ACON-A
2. Determine source of the problem and fix. When problem is resolved begin run where it was terminated.
3. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: reselect the correct table name (*e.g.*, ODP1098-1)
 - b. START LINE: begin where the error took place
 - c. END LINE: 31
 - d. MODE: NIGHT
 - e. FILENAME: should be as follows: Operators first initial, date, followed by a next letter in the sequence after the original that began with A and followed by .WKS (*e.g.*, D091701B.WKS). The entire line needs to be typed in without mistakes. You cannot edit the line it needs to be entered in one shot or press <Enter> and begin again (*e.g.*, the line should read as follows
D:\DATA\D091701B.WKS).
 - f. LIST TYPE should be FORMAT3
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: NO
4. Click SAVE to save sequence. Make sure there is plenty of paper in printer to complete the run.
5. Click MEASURE to begin run.
6. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad. Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D091701A.WKS A:). Press Ctrl and 1 (from keypad) to return to ISODAT.

Stanford University Delta_{plus}/Carlo Erba Elemental Analyzer Operation

III) Run interrupted by error

1) PART 1: Able to escape out or error

- A. If a gray window with a red bar will be displayed on the screen obstructing the normal display. Locate the source of the error (*e.g.*, high voltage out). Fix error then attempt to press ESC. If the error display disappears and the instrument appears to be working watch it carefully because it may actually be hung up and just going through the motions. It is best to just press ALT-T the run. If the error appeared a short time ago it is just a simple matter of restarting the sequence as follows
1. Click on ALT-T (upper left screen). This will exit CNF-B ACON-B
 2. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: reselect the correct table name (*e.g.*, DM041700)
 - b. START LINE: begin where the error took place
 - c. END LINE: 31
 - d. MODE: NIGHT
 - e. FILENAME: should be as follows: Operator's first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D091701B.WKS). The entire line needs to be typed in without mistakes. You cannot edit the line it needs to be entered in one shot or press <Enter> and begin again (*e.g.*, the line should read as follows
D:\DATA\D091701B.WKS).
 - f. LIST TYPE should be FORMAT3
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: NO
 3. Click SAVE to save sequence. Make sure there is plenty of paper in printer to complete the run.
 4. Click MEASURE to begin run.
 5. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad. Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D091701A.WKS A:). Press Ctrl and 1 (from keypad) to return to ISODAT.

Stanford University Delta_{plus}/Carlo Erba Elemental Analyzer Operation

2) PART 2: Unable to escape out or error

- A. If a gray window with a red bar will be displayed on the screen obstructing the normal display. If after looking for the fault and do not see one then attempt to press ESC. If the error display does not disappear to the point where the screen freezes then you will have to reboot the computer by following the reboot sequence.
1. Press Ctrl, Alt, Del to reboot computer.
 2. At the Load Real/32 prompt, Type N <Enter> this will load Win95
 3. At login prompt, Type sil <OK>
 4. Click on Scan Disk (on desktop). Let the program delete any fragments - DO NOT FIX THEM, just delete them.
 5. Restart the computer using the START button
 6. At the Load Real/32 prompt, Type Y <Enter> this will load ISODAT v7.1 allow the microprocessor to reset before doing anything (message is displayed at top of screen).
- B. Now continue as follows to prepare the run:
1. Click on CNF-A, ACON-A, 2-SEQUENCE ACQ, Click on TABLE DIR (Shift F10),
 - a. TABLE NAME: select the correct table name (*e.g.*, ODP1098-1)
 - b. START LINE: begin where the error took place
 - c. END LINE: 31
 - d. MODE: NIGHT
 - e. FILENAME: should be as follows: Operator's first initial, date, followed by a next letter in the sequence after the original that began A and followed by .WKS (*e.g.*, D091701B.WKS). The entire line needs to be typed in without mistakes. You cannot edit the line it needs to be entered in one shot or press <Enter> and begin again (*e.g.*, the line should read as follows D:\DATA\D091701B.WKS).
 - f. LIST TYPE should be FORMAT3
 - g. FILE TYPE: LOT
 - h. SAVE DATA TO DATABASE: NO
 2. Click SAVE to save sequence. Make sure there is plenty of paper in printer to complete run..
 3. Click MEASURE to begin run.
 4. To transfer the run data from hard disk to floppy disk you Press Ctrl and 2 (from the number keypad). Using DOS (*i.e.*, CD\DATA and DIR and COPY) commands go to the directory D:\DATA and copy the file to floppy disk (*e.g.*, at prompt D:\DATA> Type COPY D091701A.WKS A:). Press Ctrl and 1 (from keypad) to return to ISODAT.



Figure P1: Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M on the left to Vent-R in the middle. The oxygen vent is on the right.

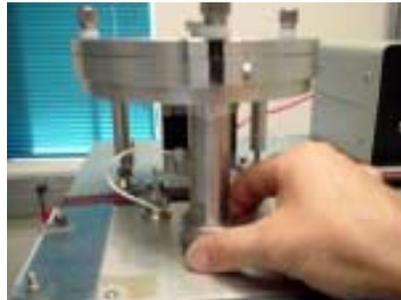


Figure P2: Removing the Costech Analytical zero blank carousel from EA reactor column. First open the purge valve on top (left photo) then loosen the connections to the reactor column (middle photo) and the helium line (right photo). Then move carousel to the side to allow removal of the quartz insert.



Figure P3: Removing quartz insert from EA reactor column. A marker pen works great at removing the insert (left photo). Once the insert is removed enough grab the top with your fingers, remove completely and place into the waste beaker (middle photo). Note the location of the carousel. In this position there is no obstruction when removing the insert. Place approximately 7 mm of quartz wool in the bottom of the insert and slide it into the reactor column (right photo).



Figure P4: Replacing the Costech Analytical zero blank carousel on to the EA reactor column. First secure the connections to the reactor column (left photo) and the helium line (middle photo). If you are not loading samples allow 2 minutes for the carousel head space to purge before closing the purge valve on top of the carousel (right photo).

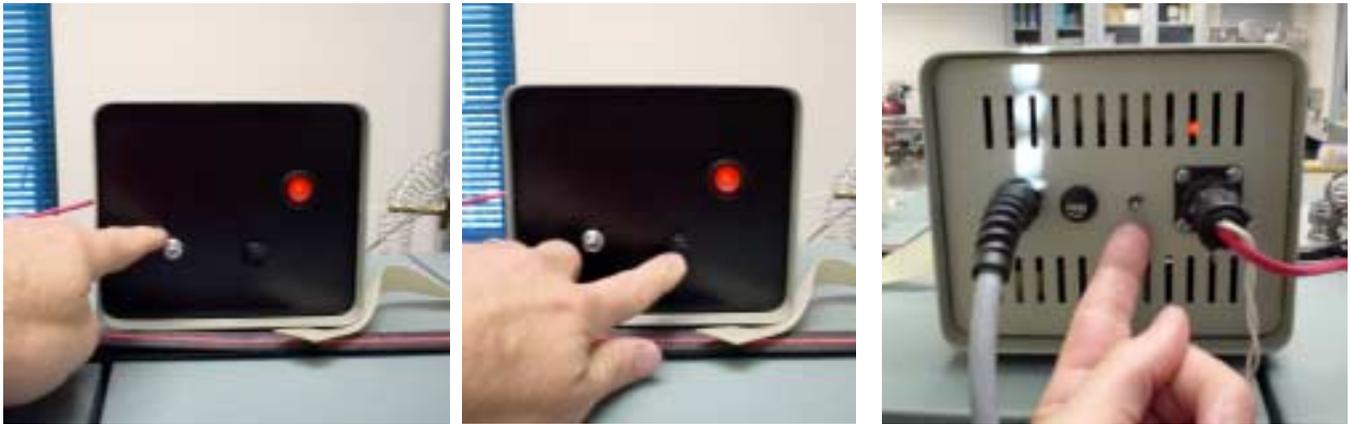


Figure P5: Costech Analytical zero blank carousel operation. Use the toggle switch to select sample drum (32 hole = up, 50 hole = down, left photo). Use button to advance carousel one position (middle photo). Use switch on rear panel to align carousel (up = advance, down = reverse, right photo).



Figure P6: Loading Costech Analytical zero blank carousel. First open the purge valve on top of the carousel (left photo). Next loosen the 3 thumbscrews (middle photo) and then open the lid to the carousel (right photo). When finished close the lid and secure with thumbscrews. Allow the carousel to purge for a minimum of 2 minutes before closing purge valve.



Figure P7: Checking and changing the magnesium perchlorate water trap on the Carlo Erba NA1500. Observe upper left portion of trap to see if magnesium perchlorate is sticking or degrading. To remove the water trap loosen the red caps on both ends of the glass trap (left and middle photo). Remove Teflon/silicon seals from both ends and check for cracking (right photo). Replace if necessary or reuse seals. Make sure silicon portion of seal seats against the glass trap and the Teflon against the stainless steel flange.

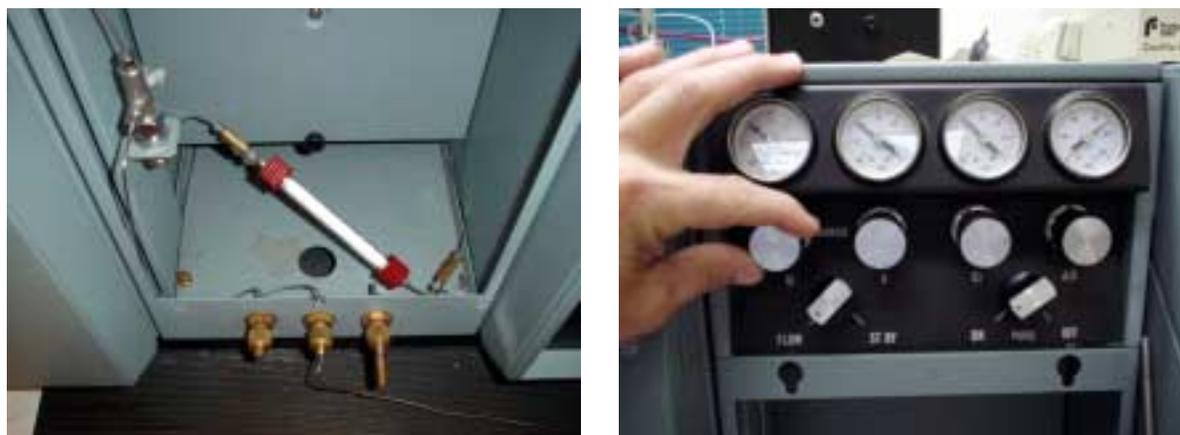


Figure P8: Leak checking the Carlo Erba NA1500. Move stainless steel line from Vent-M to Vent-R (see Fig. 1) and place plug (left photo) on Vent-M. Turn down (counter clockwise rotation) the helium pressure by using the knob labeled M on the EA (right photo). Observe the M helium gauge for leaks. When finished restore helium pressure to 90kPa (clockwise rotation).

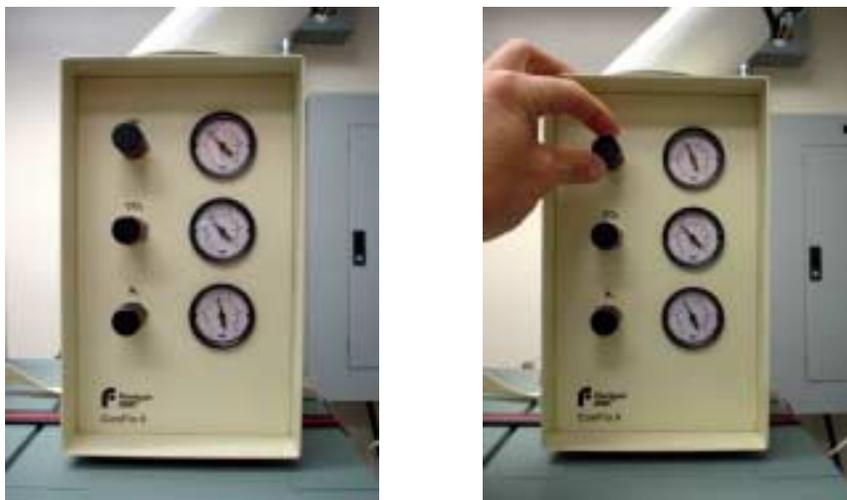
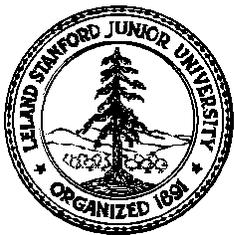


Figure P9: Select the helium dilution on the Finnigan ConFloII interface box (left photo). Depending on the samples being analyzed you may need to adjust the helium dilution prior to beginning a run (right photo).



Figure P10: Checking the base level on the Carlo Erba NA1500 prior to beginning a run. When the base level is between 660 to 725 μ V and stable switch the stainless steel line connection EA to the ConFloII interface from Vent-R to Vent-M (Fig 1). The baseline level will drop about 50-70 μ V and stabilize. This is normal because you are pulling a vacuum on the mass spectrometer fused silica capillary.



Stanford University Stable Isotope Laboratory
On-line Manual
(Prepared by David A. Mucciarone)

Section 3A: Carlo Erba NA 1500 Elemental Analyzer Procedure

I) Operation Principles for CHNS Analyses:

Elemental analyzers (Fig. 1) operate based on a flash combustion in which a sample contained within a tin or silver capsule is dropped into a combustion/reduction reactor (for CHNS analyses) held at 1020°C. This short flash combustion is accomplished when the tin capsule is exposed to a gas flow temporarily enriched with ultra high purity (UHP grade 4.3) oxygen. The resulting oxidation raise temperatures to >1700°C. The encapsulated sample, depending on its composition, will combust generating one or more of these gases: N_xO_x , CO_2 , H_2O , and SO_2 . Depending on the type of gas(es) you want to analyze, a combustion/reduction column or separate combustion and reduction columns (usually at 650°C) must be installed in the elemental analyzer furnace.

Example 1: If you want to analyze CNS you must install a single combustion/reduction column is used and would contain tungstic anhydride which is required for samples containing sulfur and halogens, and reduced copper wire for the reduction of nitrogen oxides to N_2 . N_2 , CO_2 , H_2O , and SO_2 are then moved through the column and entire system by an ultra pure helium (UHP grade 5.0) carrier gas. An adsorption trap containing magnesium perchlorate (anhydrous) must be installed for the removal of H_2O . The remaining N_2 , CO_2 , and SO_2 gases travel through a chromatographic column (Porapak PQS) and are detected with a thermal conductivity detector (TCD). See appendix for schematic diagram on NCS configuration of elemental analyzer.

Example 2: If you want to analyze only NC two columns are used. The reaction column would contain chromium oxide catalyst for oxidation and halogen removal and silvered cobaltous/cobaltic oxide for removal of SO_2 . The reduction column would contain reduced copper wires for the reduction of nitrogen oxides to N_2 . N_2 and CO_2 , and H_2O are then moved through the column and entire system by an ultra pure helium (UHP grade 5.0) carrier gas. An adsorption trap containing magnesium perchlorate (anhydrous) must be installed for the removal of H_2O . The remaining N_2 and CO_2 gases travel through a chromatographic column (Porapak PQS) and are detected with a thermal conductivity detector (TCD). See appendix for schematic diagram on CN configuration of elemental analyzer.

Example 3: If you only want N you would use the same setup in Example 2 where N_2 and CO_2 , and H_2O are carried through the columns. An adsorption trap containing soda lime or Ascarite would be added to remove the CO_2 . An adsorption trap containing magnesium perchlorate (anhydrous) must be installed for the removal of H_2O . The remaining N_2 gas travels through a chromatographic column (Porapak PQS) and are detected with a thermal conductivity detector (TCD). See appendix for schematic diagram on N configuration of elemental analyzer.

Note: Since we use the elemental analyzer coupled to the mass spectrometer (Finnigan Delta plus) via a continuous flow device (Finnigan ConFloII) we always use the water adsorption trap containing magnesium perchlorate (anhydrous) to remove the H_2O . However, if only the elemental analyzer is used and H is desired then the adsorption trap containing magnesium perchlorate (anhydrous) can be removed.

Separation of N_2 , CO_2 , H_2O , and SO_2 occurs within the GC column (4 m long with Porapak SQ, 60°C) various retention times for N_2 , CO_2 , H_2O , and SO_2 with a He carrier flow between 55 and 65 ml/min

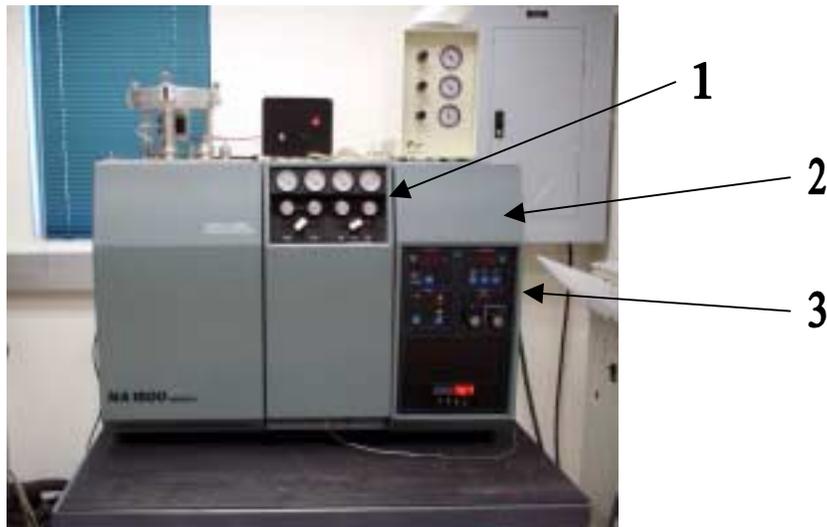


Figure 1: Carlo Erba NA1500 Series 2 Elemental Analyzer. Number 1 refers to the flow and pressure control panel, 2 refers to the instrument control panel, and 3 refers to furnace/oven temperature control panel.

(100 - 105kPa). Detection of these gases is by a thermal conductivity detector (TCD) at 190°C. After detection, N₂, CO₂, and SO₂ exit through a He vent as a function of their individual retention times.

When the elemental analyzer is coupled to the mass spectrometer, attached to the He vent is a stainless steel line connecting the EA to the ConFloII. The ConFloII is a series of regulators and valves that control the introduction of gas(es) from the EA (usually N₂ and CO₂), N₂ and CO₂ reference gases, and helium dilution for CO₂. The N₂ and CO₂ flow to the mass spectrometer through a fused silica capillary into the mass spectrometer ion source. The N₂ and CO₂ are ionized to N₂⁺ and CO₂⁺ focused with numerous plates deflected through a flight tube surrounded by an electromagnet and detected by faraday cups (collectors).

A: Elemental Analyzer Computer Operation.

Usually the computer is running the Finnigan ISODAT software. If this is the case you will need to follow Step 1 below in order to load the EAGER 100 software. However, there are other possibilities once the computer is rebooted. Observe the screen and determine which situation applies and then follow that procedure outlined below.

1. Finnigan ISODAT software running.
2. C: prompt.
3. Computer is in Win95.
4. Eager software is already running.

Depending on the condition above follow the corresponding procedure below:

1. If the Finnigan ISODAT software is running you will need to perform the following to load the EAGER 100 software.
 - a. Press and hold the ALT key then type the letters Q U I T. This will exit ISODAT and bring you to the REAL32 C:\>
 - b. You will now have to reboot the computer. Before doing this make sure you understand everything in this step b before continuing. **Do not perform any functions until Step c.** As the computer is booting up a lot of information will display on the screen. You will need to follow the screen throughout this sequence. After the screen displays the following lines

Adaptec AHA-2940 Ultra/Ultra W SCSI BIOS v1.32S4
 (c) 1997 Adaptec, Inc. All Rights Reserved
 <<< Press <Ctrl> <A> for SCSISelect (TM) Utility >>>

SCSI ID: 5 PLEXTOR CD-ROM PX-32TS

*=> "Plug and Play" peripheral -- SCSI ID auto-assigned

SCSI BIOS not installed

you will need to be prepared to press F8 (located on the upper row of the keyboard). After this last line (SCSI BIOS not installed) is displayed the following screen will be displayed.

AMIBIOS System Configuration (C) 1985-1996, American Megatrends, Inc.									
Main Processor:	P55C(MMX)				Base Memory Size:	640KB			
Math Processor:	Built-In				Ext. Memory Size:	31744KB			
Floppy Drive A:	1.44 MB 3 1/2"				Display Type:	VGA/EGA			
Floppy Drive B:	None				Serial Ports:	3F8,2F8			
AMIBIOS Date:	07/15/95				Parallel Ports:	378			
Processor Clock:	200MHz				External Cache:	512KB, Enabled			
Hard Disk(s)	Cyl	Heads	Sector	Size	LBA Mode	32Bit Mode	Block Mode	PIO Mode	
Primary Master:	14848	9	64	4111MB	LBA	On	16Sec	4	
PCI Devices:									
PCI Onboard Bridge Device					PCI Onboard USB Controller				
PCI Onboard IDE					PCI Slot 2 SCSI				
PCI Slot 3 VGA									

ROM ID: AAES045

When the above screen is displayed you will need to press F8. If successful the following menu will display on the screen. If you are not successful in you timing the following prompt LOAD Real/32 (Y or N): will display on the upper left of the screen. If this happens you will have to Ctrl, Alt, Del again.

<u>Microsoft Windows 95 Startup Menu</u>
1. Normal
2. Logged (\BOOTLOG.TXT)
3. Safe mode
4. Safe mode with network support
5. Step-by-step confirmation
6. Command prompt only
7. Safe mode command prompt only
8. Previous version of MS-DOS
Enter a choice:

At this prompt (Enter a choice) you can either enter 6 or 8 followed by <ENTER>. You will then be given the C:\> prompt. Type STARTEA <ENTER> and follow the prompts. This will load the Eager 100 software.

- c. Now that you understand Step b, continue by Pressing Ctrl, Alt, Del to reboot the computer.
- d. When the AMIBIOS System Configuration screen is displayed as described in Step b, Press F8.
- e. When the Microsoft Windows 95 Menu appears on the screen Type 6 or 8 <ENTER>.
- f. At the C:\> prompt type STARTEA <ENTER> and follow the prompts. This will load the Eager 100 software.

2. If the C:\> prompt is displayed on the screen the easiest way to rectify the situation is to type STARTEA press <ENTER> and follow the prompts. This will load the Eager 100 software
3. If in Windows 95, select START, restart computer then follow Steps 1c - 1f.
4. Nothing to do.

B: Elemental Analyzer Operation.

If the computer is ready and displaying the EAGER 100 main menu (Fig. 2) as below, then you are ready to begin setting up the elemental analyzer.

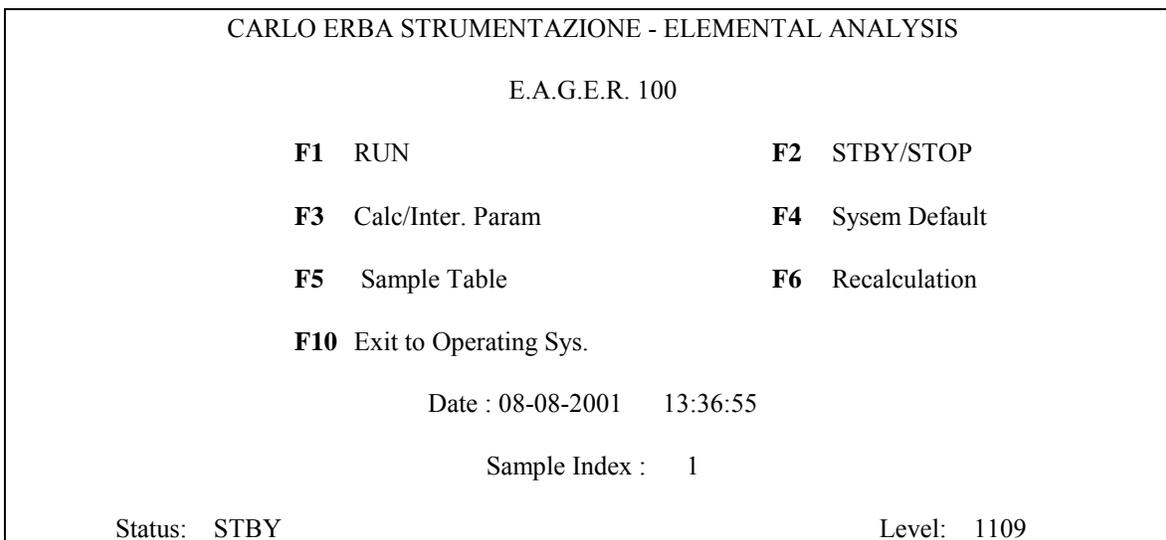


Figure 2: EAGER 100 Main Menu on Carlo Erba NA1500 Series 2 elemental analyzer. F# refers to the function keys on the top of the keyboard.

1: Temperature and Time Settings for NC analyses

See Appendix for column configuration for NC analysis. The following parameter are when using a 4 m GC column. To perform the following steps refer to Figures 3 and 4 for all buttons and dials.

1. Check to see if the filament and oven are off standby if not turn on the filament (see 11, Fig 3) and take the furnace off standby, both buttons should be up (see 10, Fig 3).
2. Make sure the furnace temperatures are set correctly. If they are not set as listed below use the respective dial(s) to the proper temperature.
 - a. Left furnace = 1020°C (see 1; Fig 3)
 - b. Right furnace = 650°C (see 2; Fig 3)
 - c. Oven = 60°C (see 3; Fig 3)

- d. Filament = 190°C (see 4; Fig 3)
 3. Make sure the run times are set properly, if they are not, use the respective dials to set them as listed below.
 - a. Cycle time = 110 seconds for C & N analyses (see 5; Fig 3)
 - b. Sample start time = 15 seconds (see 6; Fig 3)*
 - c. Sample stop time = 16 seconds (see 7; Fig 3)*
 - d. Oxygen injection stop time = 70 seconds (see 8; Fig 3)
 - e. Peak enable time = 50 seconds (see 9; Fig 3)
- *NOTE: The start and stop times can vary from 15 to 25 seconds. However, the two values should never be more than 1 second apart.

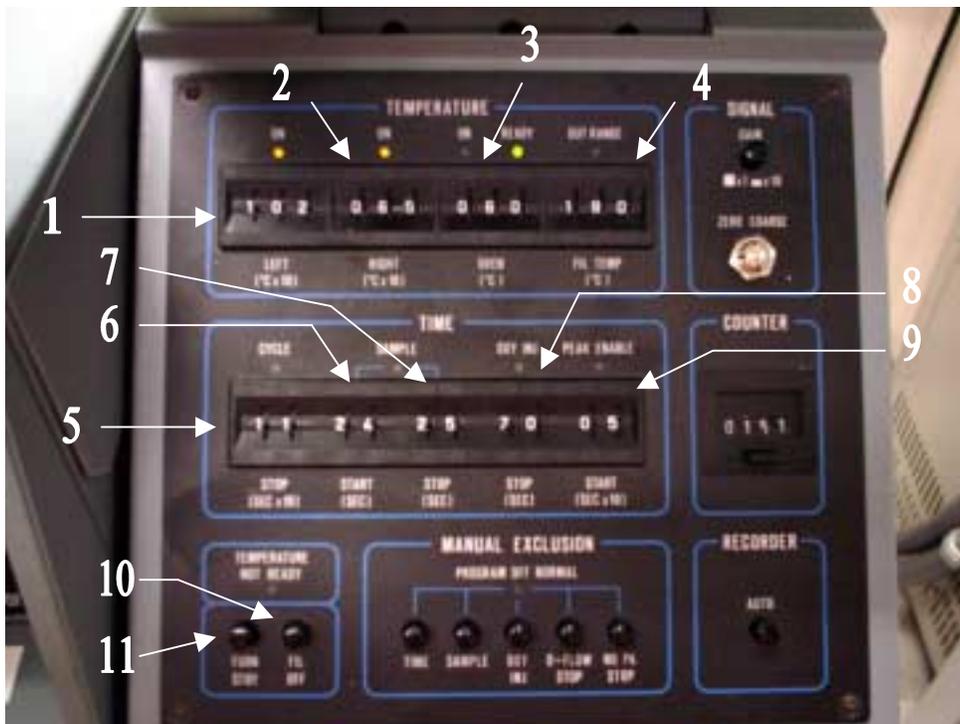


Figure 3: Instrument control panel on the Carlo Erba NA1500 Series 2 elemental analyzer. Numbers in photo are referenced in text.

4. When the EA has obtained the preset temperatures you set above the ready light (see 4, Fig 4) will illuminate.
5. To check to see if the proper temperatures are correct press buttons 2, 5, and/or 6 (Fig 4). The temperatures will be displayed above the buttons (see 3; Fig 4).

2: Gas Flow Rate Settings

It is necessary to make sure all of the flow rates are set properly. Normally nothing needs to be done however, checking these prior to a run will insure proper operation. Refer to Figure 5 when performing the following steps.

- A. Pressures and flow rates on the elemental analyzer should be set at the following:
 1. The helium flow should always be on. However, make sure helium flow is set to Flow not on Stand-by (see 1, Fig 5).

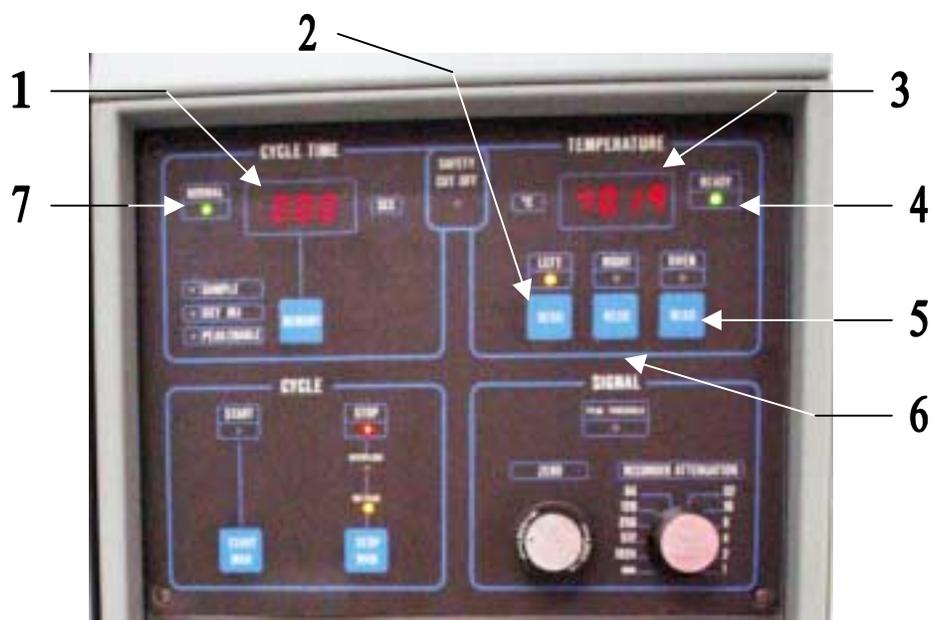


Figure 4: Cycle timer and furnace/oven temperature display panel on the Carlo Erba NA1500 Series 2 elemental analyzer. Numbers in photo are referenced in text.

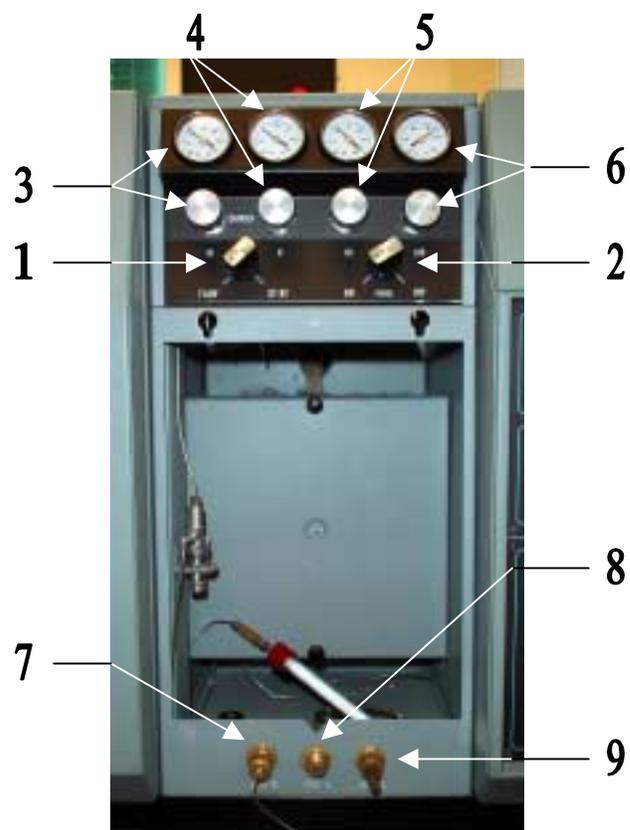


Figure 5: Regulator and flow control on the Carlo Erba NA1500 Series 2 elemental analyzer. When elemental analyzer is coupled to the Finnigan Delta plus IRMS Vent-M (7) connected to the

2. The purge should also be on. The purge line on this instrument runs to a storage flask containing magnesium perchlorate for the water trap. It is always best to make sure (see 2; Fig 5).
3. Set the Helium carrier gas and Reference pressures to 95 KPa which is approximately 55 to 65 ml/min (see 3 & 4; Fig 5).
4. Set the Oxygen pressure to 100 KPa which is about 20 to 30 ml/min (see 5; Fig 5).
5. The Air pressure should already set to 350KPa for the small volume pneumatic carousel (AS-200), the Costech Zero Blank carousel does not require air, however, the pressure should remain at 350KPa (see 6; Fig 5).

B. Pressures at the tank regulators should be set at the following:

1. Helium (UHP Grade 4.6) at 40psi.
2. Oxygen (UHP Grade 4.3) at 40psi

If for some reason the instrument was on stand-by it will take approximately 20 to 30 minutes for the left furnace to reach 1020°C from the stand-by temperature of 768°C.

C: Entering sample identification labels and weights

Most of what you will have to do with the computer is menu driven by function keys. These keys are located on the top of the keyboard labeled F1 through F10.

1. To prepare a sample table, Press <F5> (Sample Table) from the main menu (Fig. 1), then <F3> (Load Table From Disk) (Fig. 6). At the prompt (Drive name and directory <A:\xxxx>) type C:\EAGER (not case specific) <ENTER>. A list of filenames will appear on the screen. Type START <ENTER>. This will load a new sample table and clear out the old/previous runs standard values.
2. To enter labels, run type, and weights in the table <F1> (Edit Sample Array) see Figure 6. Once in the sample table enter the sample name followed by an <ENTER> (Fig. 7).
 - a. The first samples run will be conditioners which are simply standard (approx.. 0.3 to 0.6mg) folded in a tin or silver capsule. The protocol is three (3) conditioners which are Acetanilide standard. A label COND is sufficient.
 - b. Two (2) blanks are run after the standards (folded empty tin or silver capsules) and can be labeled BLANK.

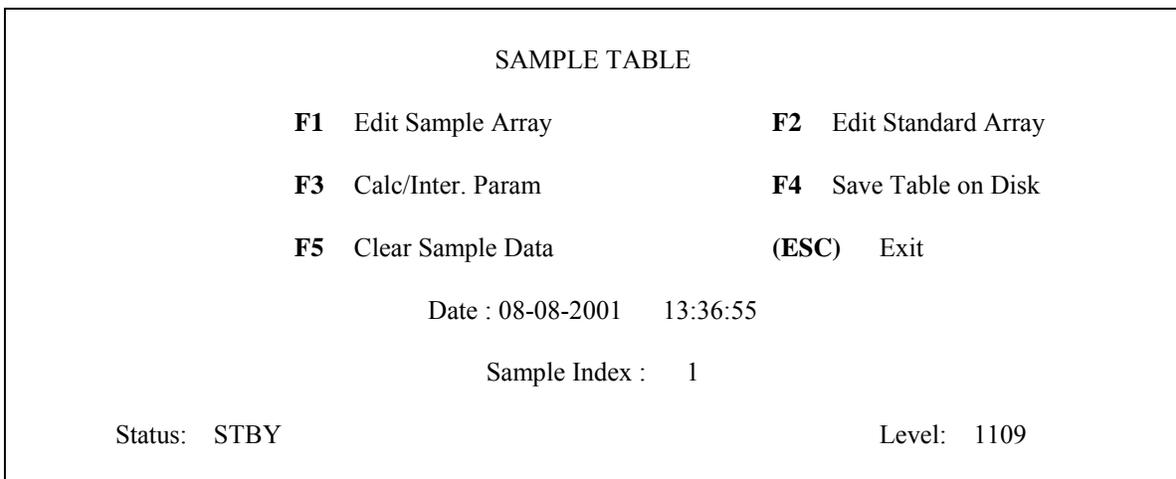


Figure 6: EAGER 100 Sample Table Menu.

- c. Three (3) standards are run next and can be labeled Acetanilide (AA is sufficient).

- d. Unknowns of course have their own identification and are run next. Since it is a tedious process of typing and there is not copy/paste function you can use abbreviations, but keep track of what you select.
2. Sample type is 3 for conditioners and unknowns, 2 for standards, and 1 for blanks, <ENTER>(Fig. 7).
3. Standard type only applies to standards (e.g., Acetanilide is 3, Sulfanilamide is 4), <ENTER>(Fig. 7).
4. Sample weight are typed in followed by <ENTER>.
5. Samples can be entered while the instrument is in operation however, you need to load all of you samples into the Costech zero blank carousel first. Note: you must exit the sample table after each sample run before the next sample will be analyzed. This should not be a problem since there is 10 minutes between runs.

SAMPLE TABLE				
#	Sample Name	Sample Type	Standard Type	Weight
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Sample Type	0=Bypass	1=Blank	2=Std	3=Unk
	F3 Calc/Inter. Param		F4 Save Table on Disk	
	F5 Clear Sample Data		(ESC) Exit	
	Date : 08-08-2001	13:36:55		
	Sample Index :	1		
Status:	STBY		Level:	1109

Figure 7: EAGER 100 Sample Table Menu.

D: Calculation and Integration Parameters

This menu routine contains all the major run parameters and can be accessed from the main menu by pressing <F3> (Fig. 2). This menu contains retention times, run time, plot attenuation, etc. (Fig. 8a-b). There are two 'pages' to this menu. All changes to the menu must be followed by <ENTER>. The following are examples of the Integration Parameters Menu:

--INTEGRATION PARAMETERS--	
Minimum Height (millivolts)	10
Inhibit Time (seconds)	10
Run Time (seconds)	525
Plot Attenuation (mV, 0=Autorange)	2
Number of Analyses	variable 1-49*
Sample Index	1
Plot on Paper (0=No, 1=Yes)	0
Report on (0=Video, 1=Printer, 2=Disk)	2
(ESC) to Exit	(ENTER) To confirm data in memory

Figure 8a: EAGER 100 Integration parameter sub-table.

--CALCULATION PARAMETERS--	
Carbon Retention Time (seconds)	350
Hydrogen Retention Time (seconds)	0 [†]
Nitrogen Retention Time (seconds)	190
Oxygen Retention Time (seconds)	0
Sulfur Retention Time (seconds)	0
Calc. Method 0=K Fact. 1=Linear Reg.	0
Protein Factor 0=No Protein calc	0
Calorific calc. 1=Solid, 2=Liquid	0
(ESC) to Exit	(ENTER) To confirm data in memory

Figure 8b: EAGER 100 Integration parameter sub-table.

*Note: The number of analyses depends on how many conditioners, blanks, standards, and samples are to be run and the sample drum used in the Costech zero blank carousel.

†Note: The hydrogen should be set to 0 if the anhydrous water trap is installed. Otherwise the standards will not calculate K factor values correctly.

E: System Default Parameters

This menu is activated by pressing <F4> from the main menu (Fig. 2). The main purpose of this menu is allow the operator the option of where to store the analytical data. Output header information can also be entered. Enter the information displayed in Figure 9. For the RAW and REP filenames it is useful to use the same for both. There is no need to add the extension to the filename this will be done automatically. You will note that in Figure 8a the parameter for plotting on paper is turned off. All of the data obtained from each run is saved on disk. When the run is completed this file will be converted into Excel and processed.

--SYSTEM DEFAULT--		
Form Feed	(N = No Y = Yes)	N
Save Raw Data on Disk	(N = No Y = Yes)	Y
Disk and Directory Name for Ray Data	(A, B, C)	C:\EAGER
Filename for Raw Data	(xxxxx.RAW)	_____*
Disk and Directory name for Report	(A, B, C)	C:\EAGER
Filename for Report	(xxxxx.REP)	_____*
Company Name	(xxxxxxxxxxxxxxxxxxxxxx)	Stanford University
Operator Name	(xxxxxxxxxxxxxxxxxxxxxx)	Stable Isotope Lab
(ESC) to Exit (ENTER) To confirm data in memory		

Figure 9: EAGER 100 System Default Menu.

***Note:** An example would be DAM1 which would end up being DAM11.RAW, DAM12.RAW, DAM13.RAW to DAM1##.RAW. There would be as many RAW files as there are runs and they would be labeled in sequence. The REP file is a report of all the runs therefore there will only be one file called DAM1.REP in this example.

F: Recalculation Parameters

This menu is activated by pressing <F6> from the main menu (Fig. 2). This menu routine allows the operator to change K factor and Blank values (Fig. 10a). For example if one standard was out of line with the others you could manually recalculate the standard average and enter the new value(s) in the table. When satisfied with the new values press <ESC> and enter which samples you want the new K factors to effect (Fig. 10b).

The following are the equations used to calculate K Factors and Weight percent.

$$K \text{ Factor} = (Wt. \text{ std} * \%Tstd) / (Area \text{ std} - Area \text{ blk})$$

- Where: Wt. std = weight of standard (mg)
- %Tstd = theoretical percentage of standard
- Area std = peak area or integral of standard
- Area blk = peak area or integral of blank

$$Weight \text{ percent} = (K \text{ Factor} * (Area \text{ unk} - Area \text{ blk})) / (Wt. \text{ unk})$$

- Where: K Factor = average K Factor value
- Area unk = peak area or integral of unknown
- Area blk = peak area or integral of blank
- Wt. unk = weight of unknown (mg)

G: Data Conversion and Transfer

When the run is complete you will need to convert the REP file into a DBF file. The REP file is an ASCII file that is designed to output to a printer not a file so it is necessary to convert this to a more useful format. The Eager 100 software has a program that converts the REP file to a database 3 formatted file or DBF. A VBASIC program is then used to convert the DBF file into an Excel (XLS) file for post processing and data management. The following steps will help you through this process.

1. Press <F10> (Exit to Operating sys., Fig. 2) At the prompt (Press Y to confirm or any other key to renounce:) Type Y <ENTER>. This will allow you to exit the Eager 100 software and bring you to the C:\EAGER> MS-DOS prompt.
2. At the C:\EAGER> prompt, type DB3CONV <ENTER> and follow the prompts. This will start the database conversion program.
3. Press <F1> to Start program.
4. At the prompt PLEASE GIVE INPUT FILENAME (xxxxxxx.REP) == => Type filename.REP used <ENTER> (e.g., DAM1 do not add the .REP).
5. At the prompt PLEASE GIVE DISK NAME FOR INPUT FILENAME == => Type C <ENTER>.
6. At the prompt PLEASE GIVE OUTPUT FILENAME (xxxxxxx.DBF) == => Type same as input filename used <ENTER> (e.g., DAM1 do not add the .DBF).
7. Review the screen to make sure the source and destination are correct, e.g., the line should read:

```

SOURCE C:DAM1.REP          DESTINATION C: DAM1.DBF

PLEASE GIVE CONFIRMATION (Y - N) == => Type Y <ENTER>

```

8. When the program finishes you will return to the C:\EAGER> prompt. Insert a diskette into the floppy drive and copy the following REP and DBF files from the C drive to the A drive, see the following example. At the C:\EAGER> prompt type COPY DAM1.REP A: <ENTER> then type COPY DAM1.DBF A: <ENTER>
9. To continue running the Eager 100 software and the elemental analyzer type RLDEAGER.
10. If finished running the elemental analyzer as a stand-alone unit simply reboot the computer by pressing Ctrl, Alt, Del. The computer will eventually prompt you with LOAD Real/32 (Y or N)? Type Y, this will load the Finnigan ISODAT software.

```

--K-FACTORS--

#           K Factor Average   Blank Average   Peak Name
1             0                 0                Carbon
2             0                 0                Hydrogen
3             0                 0                Nitrogen
4             0                 0                Oxygen
5             0                 0                Sulfur

(ESC) to Exit   (ENTER) To confirm data in memory   (F1) Cursor Down
Average calculation will restart from first calibration given 0 to factors

```

Figure 10a: EAGER 100 Recalculation sub-menu

```

--RECALCULATION--

(RETURN) for sample in memory or index of sample (1 - 200)

```

Figure 10b: EAGER 100 Recalculation Sub-menu.

H: Carousel Operation, Changing Inserts, and Loading Samples

1. Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M to Vent-R (Fig. 11).
2. Remove quartz insert from EA reactor column:
 - a. Loosen purge valve on top of carousel (Fig. 12).
 - b. Next open the purge valve on top of the carousel then loosen the fittings connecting the carousel to the reactor column and helium input line. Move carousel to the side so the insert can be removed without obstruction (Fig. 12).
 - c. Carefully remove insert from reactor. Care should be taken when handling the insert because it is at 1020°C. Place hot insert into waste beaker (Fig. 13).
3. Install new quartz insert into EA reactor column:
 - a. Put approximately 7mm of quartz wool into the bottom of the insert (Fig. 13).
 - b. Carefully slide the quartz insert into reactor column until it stops (do not push or force insert, Fig. 3). The insert should stop approximately 5mm above top of reactor column). If the insert falls below the top of the reactor column add an appropriate amount of quartz wool to the reactor column (not the insert) then slide the insert in again.
 - c. With the insert in the proper position, gently push the insert down until it is flush with the top of the reactor column (do not force).
 - d. Reposition the carousel onto the reactor column and secure both connections at the reactor column and the helium carrier line (Fig. 14).
4. Carousel Operation (Fig. 15):
 - a. Front panel: Toggle switch up = 32 hole sample drum
Toggle switch down = 50 hole sample drum
Black push button = advances drum forward 1 position
Round switch up = on (red light on)
Round switch down = off (red light off)
 - b. Rear panel: Switch on the rear panel is to align the carousel after swapping out sample drums.
Toggle switch up = advance sample drum by steps
Toggle switch down = reverse sample drum by steps
5. Load samples into carousel (Fig. 16):
 - a. Loosen the 3 thumbscrews on the top of the carousel (loosen back two first then front) and fold out of the way. Flip back the carousel lid to expose sample drum.
 - b. Using forceps carefully load samples into sample drum. If first sample is not in hole 1, record start position on sample log sheet. This will be helpful if there is a problem with the run (e.g., sample capsule wedges in hole and does not drop, samples need to be removed).
 - c. Once samples are loaded, close lid and secure using the 3 thumbscrews. Tighten back two first then front.
 - d. Allow carousel to purge for 2 minutes before closing purge valve on the top of carousel lid.



Figure 11: Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M on the left to Vent-R in the middle. The oxygen vent is on the right.

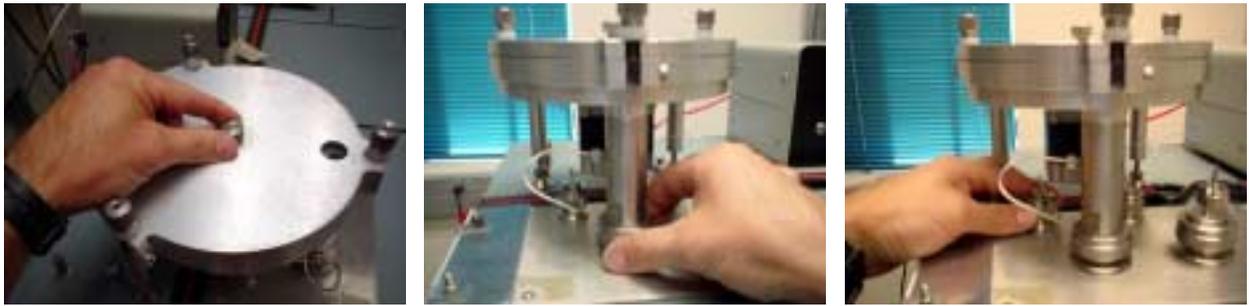


Figure 12: Removing the Costech Analytical zero blank carousel from EA reactor column. First open the purge valve on top (left photo) then loosen the connections to the reactor column (middle photo) and the helium line (right photo). Then move carousel to the side to allow removal of the quartz insert.

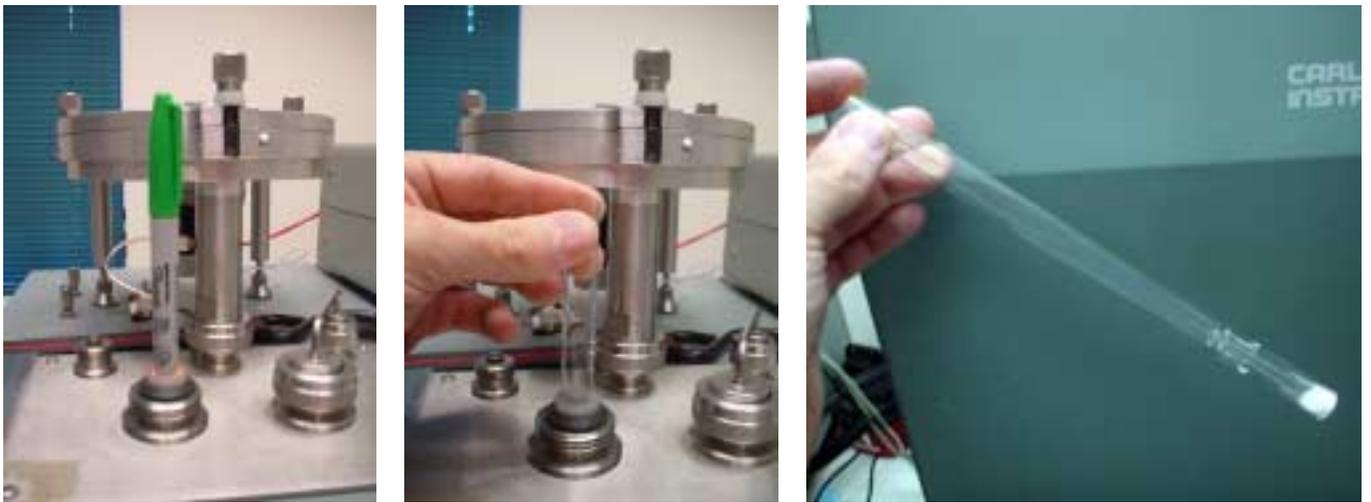


Figure 13: Removing quartz insert from EA reactor column. A marker pen works great at removing the insert (left photo). Once the insert is removed enough grab the top with your fingers, remove completely and place into the waste beaker (middle photo). Note the location of the carousel. In this position there is no obstruction when removing the insert. Place approximately 7 mm of quartz wool in the bottom of the insert and slide it into the reactor column (right photo).



Figure 14: Replacing the Costech Analytical zero blank carousel on to the EA reactor column. First secure the connections to the reactor column (left photo) and the helium line (middle photo). If you are not loading samples allow 2 minutes for the carousel head space to purge before closing the purge valve on top of the carousel (right photo).

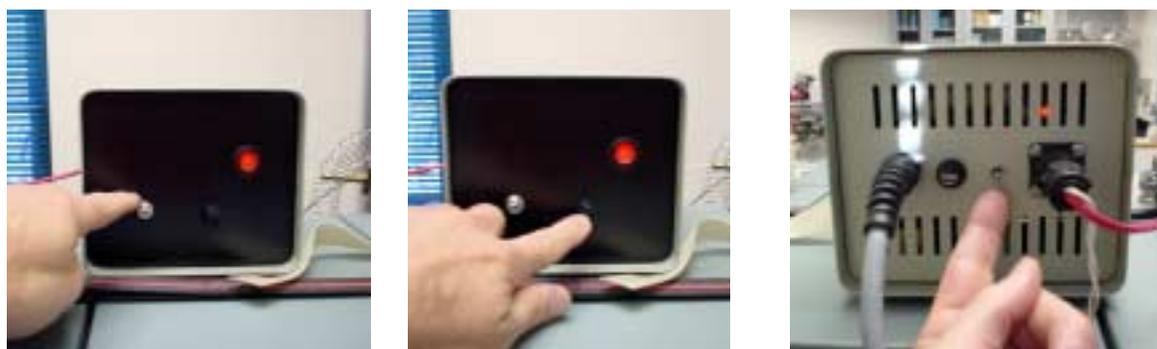


Figure 15: Costech Analytical zero blank carousel operation. Use the toggle switch to select sample drum (32 hole = up, 50 hole = down, left photo). Use button to advance carousel one position (middle photo). Use switch on rear panel to align carousel (up = advance, down = reverse, right photo).

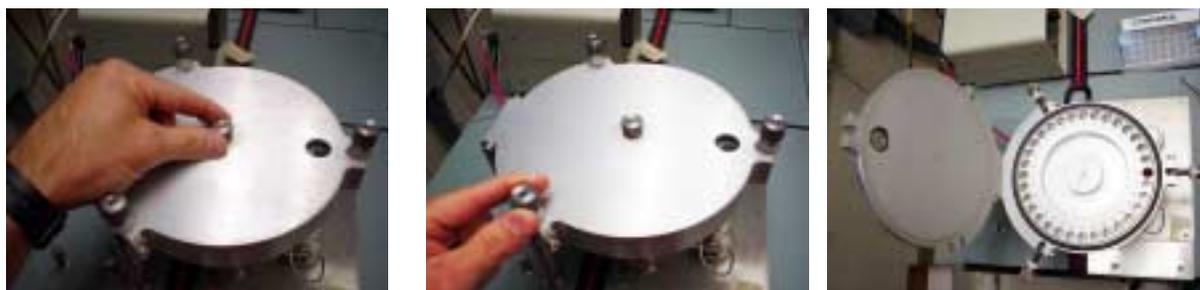


Figure 16: Loading Costech Analytical zero blank carousel. First open the purge valve on top of the carousel (left photo). Next loosen the 3 thumbscrews (middle photo) and then open the lid to the carousel (right photo). When finished close the lid and secure with thumbscrews. Allow the carousel to purge for a minimum of 2 minutes before closing purge valve.

I: Servicing Magnesium Perchlorate Water Trap and Leak Checking

1. Check the magnesium perchlorate water trap. Change when the upper 10 to 15mm of magnesium perchlorate granules appear fused together (Fig. 17).
 - a. To change loosen red caps on both ends of trap and remove. Teflon/silicon seals usually stick to the glass trap, remove seals, check for cracks and replace if necessary otherwise clean and use.
 - b. Place 5 to 7mm of quartz wool in one end of a clean trap (located in oven) and fill with magnesium perchlorate granules and gently tap trap on bench top to compact granules. Be sure to leave approximately 5 to 7mm of space in order to insert quartz wool into the open end.
 - c. Clean both faces of trap and reassemble. Place seals in red caps with Teflon portion of seal against the metal flange and secure finger tight.
 - d. Magnesium perchlorate from used trap needs to be removed and placed into the waste container. Glass trap can then be cleaned with water and placed into the oven to dry.
2. Perform a leak check EA (Fig. 18).
 - a. Place a plug over Vent-M, release pressure on M helium regulator on EA (turn black/silver knob counterclockwise).
 - b. If there is a leak the pressure on the M helium gauge will decrease. If this occurs check for leaks. The most common source of leaks will be at the carousel connection to the reactor column, seals in

- water trap, or the carousel lid seal. Make sure to restore helium pressure to 90kPa before checking for leaks. Repeat 18a and 18b if necessary until leak is repaired.
- c. If there is no leak, remove plug on Vent-M and restore helium pressure to 90kPa.



Figure 17: Checking and changing the magnesium perchlorate water trap on the Carlo Erba NA1500. Observe upper left portion of trap to see if magnesium perchlorate is sticking or degrading. To remove the water trap loosen the red caps on both ends of the glass trap (left and middle photo). Remove Teflon/silicon seals from both ends and check for cracking (right photo). Replace if necessary or reuse seals. Make sure silicon portion of seal seats against the glass trap and the Teflon against the stainless steel flange.

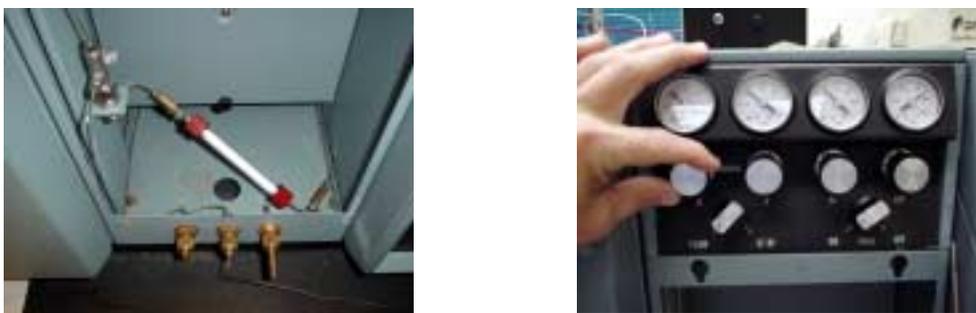


Figure 18: Leak checking the Carlo Erba NA1500. Move stainless steel line from Vent-M to Vent-R (see Fig. 11) and place plug (left photo) on Vent-M. Turn down (counter clockwise rotation) the helium pressure by using the knob labeled M on the EA (right photo). Observe the M helium gauge for leaks. When finished restore helium pressure to 90kPa (clockwise rotation).

J: Begin Analyses

ConFloII interface is connected to the EA via a stainless steel tube. When the elemental analyzer is used as a stand-alone instrument this stainless steel tube must be switched from Vent-M (see 7, Fig. 5) to Vent-R (see 8, Fig. 5). Vent-O (see 9, Fig. 5) is always open.

At the bottom of the main menu (Fig. 2) you will find 3 important status parameters. Sample Index, Status, and Level. Make sure the sample index corresponds with the position of the carousel, the status should be STBY and the level around 1050 to 1150. Make sure the switch box behind the back right corner of the EA is switched to B (Fig. 19). Once you are confident all of the various run parameters and temperatures are correct and the ready light is illuminated on the front panel of the instrument (see 4, Fig. 4) you can begin the sample run by pressing <F1>. When the instrument starts you will hear a valve click which is the oxygen injection and then the clock on the front panel will start (see 1, Fig. 4). At the sample start time (see 6, Fig. 3) you will hear the carousel rotate, at the time set for the oxygen injection (see 8, Fig. 3) you will hear the valve close, and at the end of the run time (see 5, Fig. 3) the clock will reset to 000.

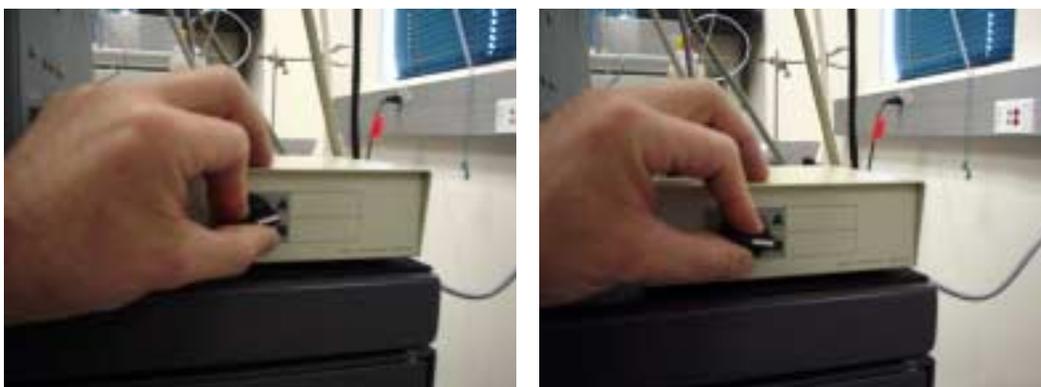


Figure 19: Switching software control between ISODAT (position A, left photo) and EAGER 100 (position B, right photo). Remember to turn the switch back to position A when rebooting back to ISODAT.

The analysis can be stopped at any time by pressing <F2> from the main menu either once (run will terminate at end of sample analysis) or twice (run will stop). This is handy if you encounter some kind of problem, however, do not restart the instrument until the counter on the front panel of the instrument reads 000 (See 1, Fig. 4).

When finished do not place elemental analyzer on stand-by, the instrument should always be in ready mode unless service of maintenance is required. Putting the instrument into stand-by mode with the Delta plus IRMS and ConFloII interface connected to either Vent-M or Vent-R could cause the helium carrier flow to the ion source to drop low enough to allow atmospheric gases to enter the open split of the ConFloII and contaminate the ion source.

As explained above, attached to the elemental analyzer is a mass spectrometer that enables us to analyze N_2 and CO_2 gas generated in the combustion/reduction reactor for $\delta^{15}N$ and $\delta^{13}C$ analysis. Configuration of the combustion/reduction reactor along with other parameters such as oven temperatures, flow rates, retention times are presented in Table 1. Do not alter these parameters. The METHODS in ISODAT are setup according to these parameters especially peak jumping. Please note that shorter columns can be used which will decrease the total run time, peak width, and separation between peaks. Higher flow rates can also be achieved with shorter columns because there is less back pressure. Changing the column temperature will also affect peak shape and retention times. When this is done the METHODS in ISODAT will need to be altered to work with these changes.

Table 1.--Set up parameters for N and C analysis using the Carlo Erba Na1500 Series 2 elemental analyzer and a 4 m GC column.

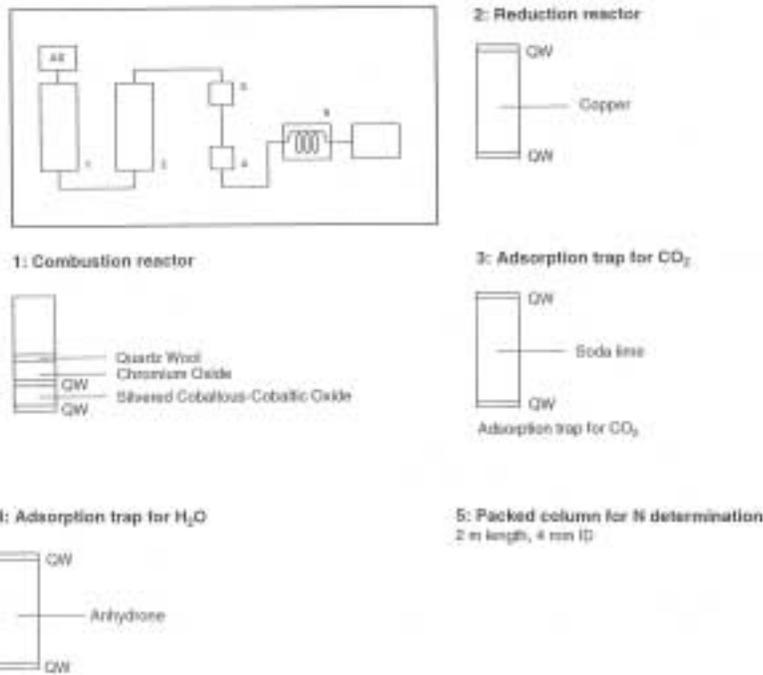
Instrument Temperatures (°C)	
Combustion oven	1020
Reduction oven	650
Chromatographic column	60
Filament	190
Flow Rates (ml min ⁻¹)	
Helium Grade 4.6, Zero (95 - 105kPa)	55-65
Oxygen Grade 5, UHP (100 - 105kPa)	20-25
Element Retention Times (seconds)	
Nitrogen as N ₂	205-220

Carbon as CO ₂	390-410
Additional Parameters (seconds)	
Cycle time	600
Sample time begin/end Costech Zero Blank carousel	15 & 16
Sample time begin/end Carlo Erba AS-200	15 & 60
Oxygen injection	65
Run time for N and C	620s (10.5 min)
Column Configuration (cm)	
Quartz combustion column (l x w)	45.0 x 1.8
From bottom to top:	
-Quartz wool	4.0
-Silvered cobaltic oxide (Co ₃ O ₄ + Ag)	6.0
-Quartz wool	0.5
-Chromium oxide (Cr ₂ O ₃)	10.5
-Quartz wool	0.5
Quartz reduction column (l x w)	45.0 x 1.8
From bottom to top:	
-Quartz wool	4.0
-Reduced copper wires (approx. 150g)	30.0
-Quartz (silica) chips	9.0
-Quartz wool	1.5

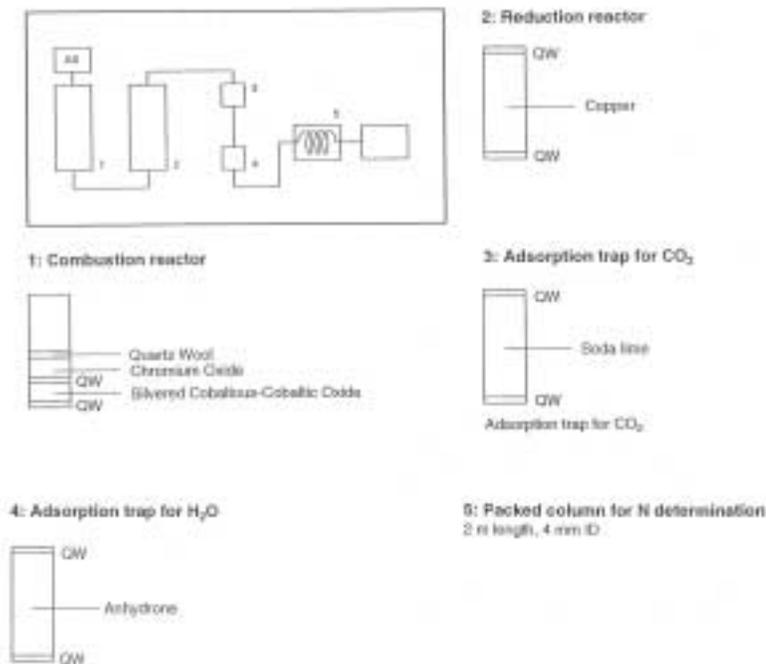
K:Summary of Operation

- 1) Move Vent-M (see 7, Fig. 5) to Vent-R (see 8, Fig. 5). This will separate the Carlo Erba NA1500 from the Finnigan Delta plus.
- 2) Check instrument run parameters as in Table 1 above.
- 3) Press <F5> (Sample Table)
- 4) Press <F3> (Load Table from Disk).
- 5) Type C:\EAGER <ENTER>
- 6) Type START <ENTER> to load new sample table.
- 7) Press <F1> (Edit sample array).
- 8) Press <F4> (Save table on disk)
- 9) Press <ESC> to exit.
- 10) Press <F3> (Calc/Integ. Param). Modify list to correspond to instrument parameters.
- 11) Press <ESC> to main menu.
- 12) Load samples into carousel. Once you have checked the baseline level to be better than 1150 and stable you are ready to begin. Press <F1> (Run).

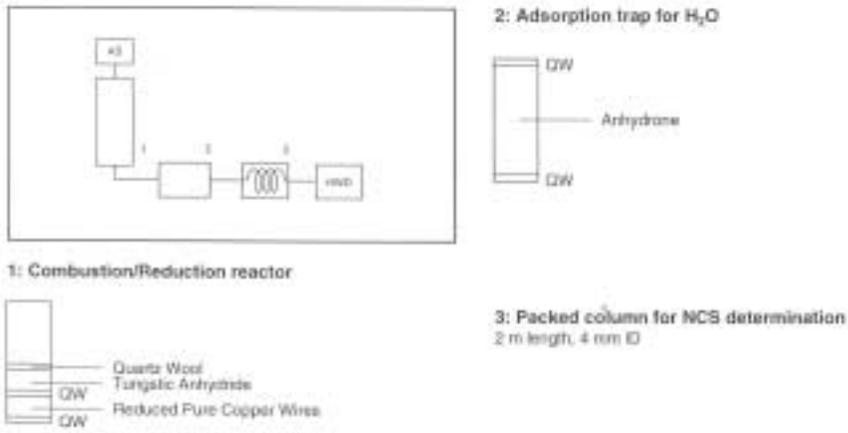
Part L: Appendix Various elemental analyzer configurations



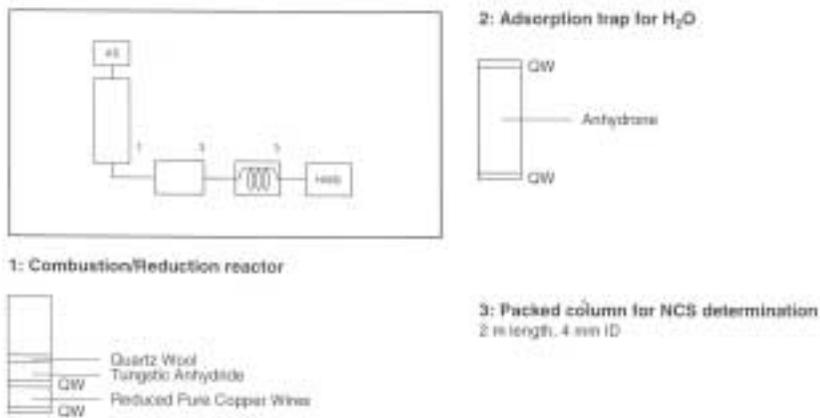
Appendix Figure 1: Nitrogen only configuration on Carlo Erba NA1500 Series 2 elemental analyzer



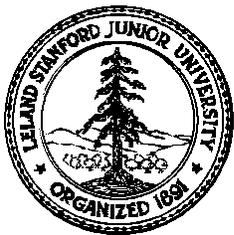
Appendix Figure 2: Nitrogen and carbon configuration on Carlo Erba NA1500 Series 2 elemental analyzer



Appendix Figure 3: Nitrogen, carbon, and sulfur configuration on Carlo Erba NA1500 Series 2 elemental analyzer



Appendix Figure 4: Sulfur only configuration on Carlo Erba NA1500 Series 2 elemental analyzer



Stanford University Stable Isotope Laboratory
On-line Manual
(Prepared by David A. Mucciarone)

Section 3B: Preparation of Elemental Analyzer Reduction (Cu) Column

This procedure is designed to prepare a reduction (copper, Cu) column for the Carlo Erba NA1500 Series II elemental analyzer (EA). The entire procedure should be performed over a secondary container, such as a tray, to capture any spilled materials. Safety glasses, rubber gloves, face mask, and a lab coat/apron are required throughout the acid preparation.

Unlike the oxidation column on the EA the reduction column needs to be changed approximately every 500 samples. This can best be determined by monitoring the mass 30/28 ratio from the Finnigan Delta_{plus} mass spectrometer. When the 30/28 ratio becomes greater than 1000 on the IAEA-N1 standard the column is close to exhaustion and needs to be closely monitored. At 5000 it is time to change the reduction column or the precision of $\delta^{15}\text{N}$ values will degrade. If $\delta^{15}\text{N}$ or %N are not desired you can run approximately 100 to 150 more sample as $\delta^{13}\text{C}$ and %C only. The purpose of this section is to serve as a guide, you should not attempt this unless you have been trained and have a good understanding on operating the EA and the mass spectrometer. Before you begin preparing the new Cu column, you should first clear an area on the bench top, obtain and organize all of the materials and equipment listed below.

Materials: Costech Analytical grade pure copper (Cu) at 0.3mm mesh wire, quartz turnings, quartz wool, and one 1.8 cm x 45 cm quartz tube (Fig. P1).

Equipment: Pliers, funnel, template, ruler, push rod, and tray (Fig. P1).

1. Switch the stainless steel line from Vent-M to Vent-R (Fig. P2). Turn down the left (oxidation) oven from 1020°C to 720°C and the right (reduction) oven from 650°C to 250°C (Fig. P3). Allow both ovens to cool before removing the reduction column. While the ovens are cooling you can prepare a new reduction column. **Note:** The reduction oven may only come down to the 300°C range because of the high temperature on the oxidation oven. **Caution:** Do not reduce the oxidation oven below 720°C because the quartz tube may become brittle and fail.
2. We have two types of quartz tubes, opaque and clear. It is easy to observe the packing and measure the amount of material added to a clear tube but more difficult to do the same on the opaque tubes. When using the opaque tubes the push rod and template will assist you in determining the amount of material in the tube.
3. Insert 1.5 to 2.0 cm of quartz wool into the bottom of the quartz reduction tube (Fig. P4). Place bottom of tube against the tray and use the push rod to tamp down the quartz wool (Fig. P4).
4. Using a funnel add approximately 2.5 to 3.0 cm of quartz turnings to the quartz tube (Fig. P4). Gently tap the tube to compact the quartz turnings. It is necessary to insure good packing of all reagents added to the quartz tube or separations will occur inside the tube when the column is heated to 650°C. Use the push rod as a measuring device against the template. The total accumulation of both quartz wool and turnings should be 5 cm.
5. Again using a funnel add 29 to 30 cm of pure Cu wires to the quartz tube (Fig. P4). As you add the Cu to the tube gently tamp the tube to compact the Cu to avoid separation when the column is heated to 650°C. Use the push rod to measure the amount of Cu added.
6. Add approximately 9.0 to 9.5 cm of quartz turnings on top of the Cu and plug the rest of the open tube with quartz wool (Fig P4).
7. Install a black sealing ring at the top with the flat portion down (Fig P5).
8. Clean the outside of the reduction tube with a Kimwipe and 2-propanol and set the tube aside (Fig P5).
9. When the oven has cooled and the new reduction column is ready you can begin removing the used reduction column.

10. Remove the furnace cover to expose the bottom of the reduction column (Fig. P6).
11. Remove both braces at the bottom of the oxidation and reduction columns (Fig. P7).
12. Completely loosen the fittings on the bottom of the reduction column (right) and only loosen the fitting on the bottom of the oxidation column (left) enough so you can swing the coupling to the side (Fig. P7).
13. Remove the bottom sealing ring, washer, and nut from the reduction column (Fig. P7).
14. Loosen and remove the fitting at the top of the reduction column (Fig P8).
15. Carefully remove the used reduction column and install the new one (see caution note in Fig. P8).
16. Assemble in reverse order. Attach the top fitting first and then install the bottom nut, washer, and sealing ring on the bottom. Swing the coupling into place and secure both fittings (Fig. P8).
17. Install both braces (Fig. P7) and perform a leak check EA (Fig. P9).
 - a. Place a plug over Vent-M, release pressure on M helium regulator on EA (turn black/silver knob counterclockwise).
 - b. If there is a leak the pressure on the M helium gauge will decrease. If this occurs check for leaks. The most common source of leaks will be at the carousel connection to the reactor column, seals in water trap, or the carousel lid seal. Make sure to restore helium pressure to 90kPa before checking for leaks. Repeat 9 and 18b if necessary until leak is repaired.
 - c. If there is no leak, remove plug on Vent-M and restore helium pressure to 90kPa.
18. Turn up the oxidation oven to 1020°C and the reduction oven to 650°C (Fig. P3). When the ready light on the front panel illuminates the instrument is ready for use.



Figure P1: Material required to prepare reduction column on the Carlo Erba NA1500 Series II elemental analyzer.



Figure P2: Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M on the left to Vent-R in the middle. The oxygen vent is on the right.

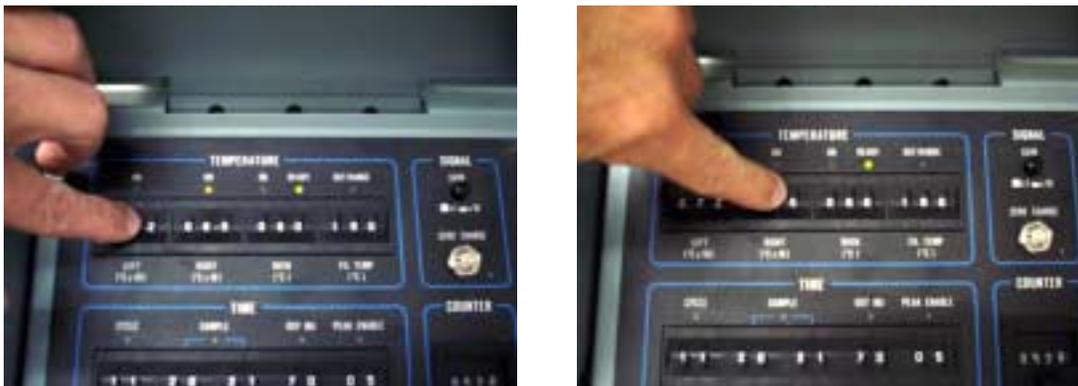


Figure P3: Turn down the oxidation oven from 1020°C to 820°C and the reduction oven from 650°C to 250°C. Allow the ovens to cool before removing the reduction column. You will have plenty of time to prepare a new column. **Note:** The reduction oven may only come down to the 300°C range because of the high temperature on the oxidation oven. **Caution:** Do not reduce the oxidation oven below 720°C because the quartz tube may become brittle and fail.

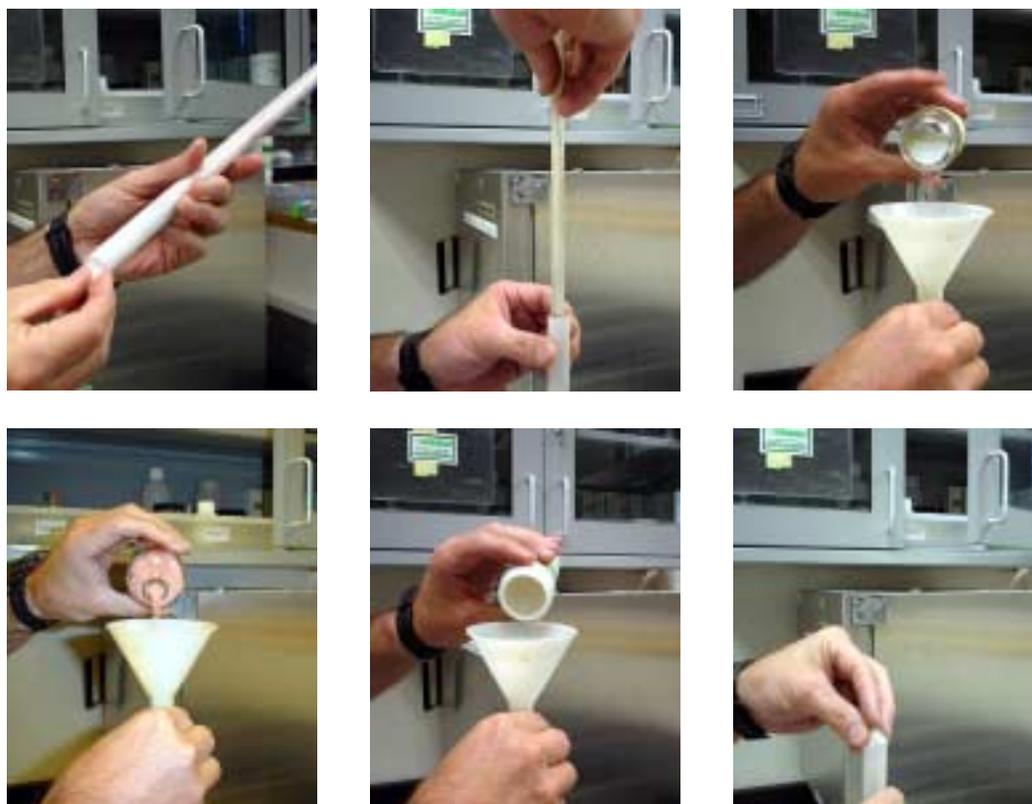


Figure P4: Preparing reduction column. Insert 1.5 to 2 cm of quartz wool into the bottom of the quartz tube (upper left photo). Gently tamp the quartz wool with the push rod (upper middle photo). Then add 2.5 to 3 cm of quartz turnings (upper right photo) for a combined total of 5 cm. Add 29 to 30 cm of pure copper wires on top of the quartz turnings (bottom left photo). Add 9 to 9.5 cm of quartz turnings on top of the copper wires (bottom middle photo) and finish with 1 to 1.5 cm of quartz wool (bottom right photo). The entire length of the reduction tube is 45 cm.



Figure P5: Install sealing ring on top of the reduction column (left photo). Clean the outside of the reduction column using a Kimwipe and 2-propanol.



Figure P6: Before you can service the columns you need to remove the oven cover to the elemental analyzer. To accomplish this slide the cover up until it stops then pull it toward you.

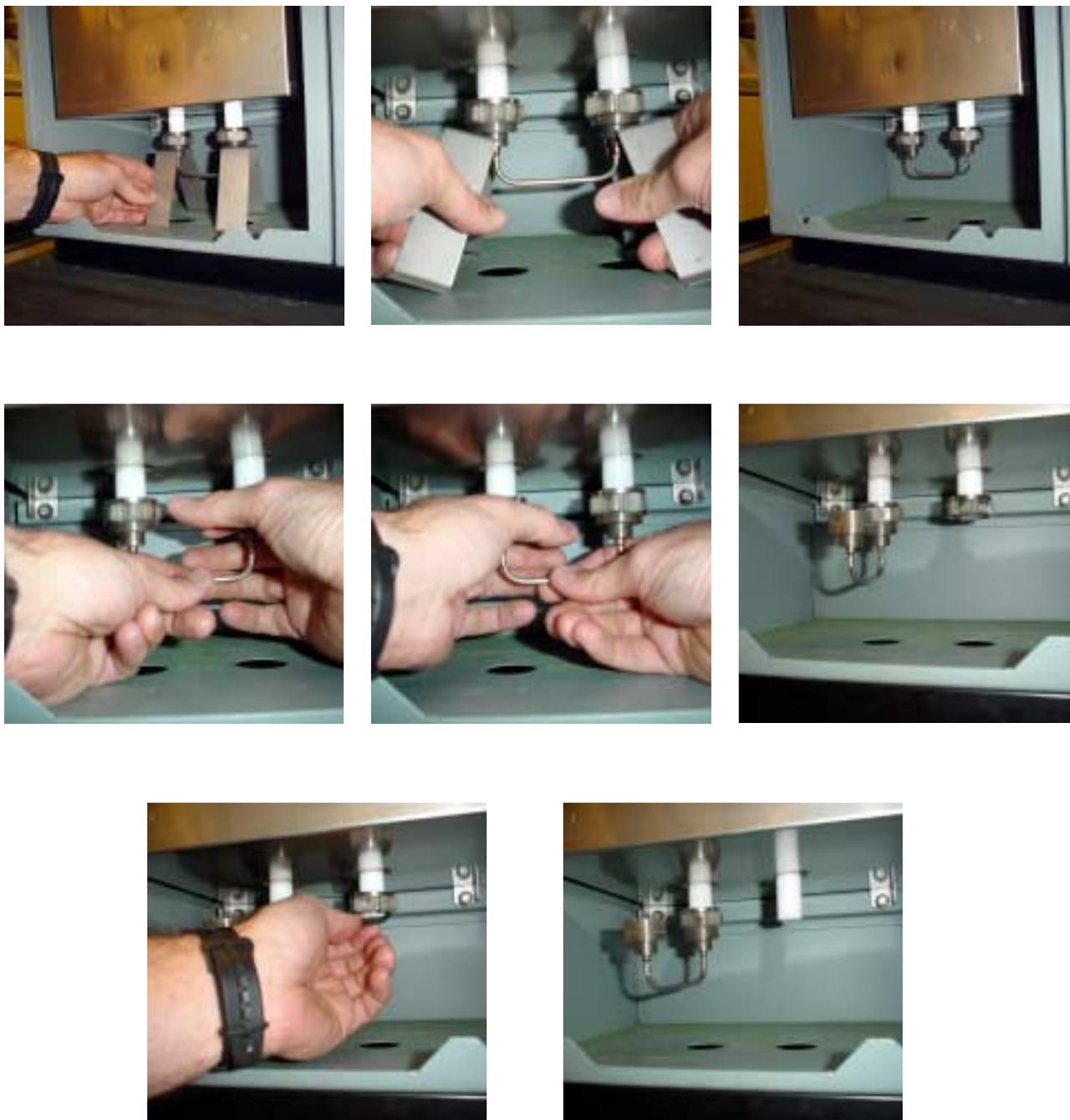


Figure P7: Disassemble the coupling joining the bottom of the oxidation and reduction columns (upper right photo). Remove both supports (upper middle photo) to expose the coupling (upper right photo). Completely loosen the fitting to the reduction column (middle left photo) then just loosen the fitting to the reduction column (center photo) and swing the coupling forward out of the way (middle right photo). Remove the sealing ring, washer, and nut from the bottom of the reduction column (bottom left photo) so that the column is free (bottom right photo).

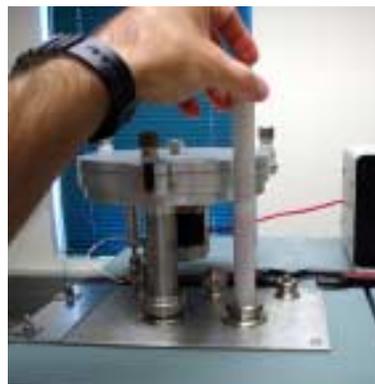
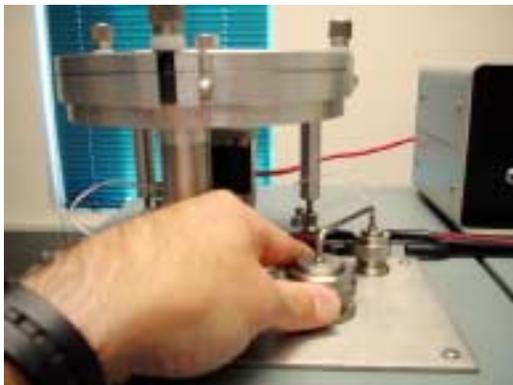
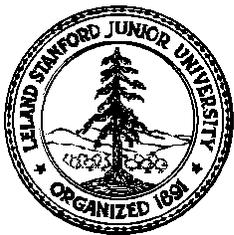


Figure P8: Remove coupling on top of reduction column. Completely loosen both fittings on top of the reduction column (upper right and left photos). Remove coupling (bottom left) to expose reduction column and carefully remove the column (bottom right photo). **Caution:** Handle with care, the reduction column is still hot around 250°C to 300°C. Place upright in a beaker until cool.



Figure P9: Leak checking the Carlo Erba NA1500. Move stainless steel line from Vent-M to Vent-R (see Fig. 2) and place plug (left photo) on Vent-M. Turn down (counter clockwise rotation) the helium pressure by using the knob labeled M on the EA (right photo). Observe the M helium gauge for leaks. When finished restore helium pressure to 90kPa (clockwise rotation).



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Section 3C: Preparation of Elemental Analyzer Oxidation Column

This procedure is designed to prepare an oxidation column for the Carlo Erba NA1500 Series II elemental analyzer (EA). The entire procedure should be performed over a secondary container, such as a tray, to capture any spilled materials. Safety glasses, rubber gloves, face mask, and a lab coat/apron are required throughout the acid preparation.

Unlike the reduction column on the EA the oxidation column is changed on a 4:1 ratio, i.e, 4 reduction columns to 1 oxidation column, which is approximately every 2000 samples. This ratio was set because at 1020°C the quartz tube begins to weaken and beyond 4:1 there is a greater risk to tube failure. The reagents in the oxidation column are still good and can be removed and reused. The purpose of this section is to serve as a guide, you should not attempt this unless you have been trained and have a good understanding on operating the EA and the mass spectrometer. Before you begin preparing the new oxidation column, you should first clear an area on the bench top, obtain and organize all of the materials and equipment listed below.

Materials: Costech Analytical chromium (III) oxide, silvered cobalt (II, III) oxide, quartz wool, and one 1.8 cm x 45 cm quartz tube (Fig. P1).

Equipment: Funnel, template, ruler, push rod, and tray (Fig. P1).

1. Switch the stainless steel line from Vent-M to Vent-R (Fig. P2). Turn down the left (oxidation) oven from 1020°C to 320°C and the right (reduction) oven from 650°C to 250°C (Fig. P3). Allow both ovens to cool before removing the oxidation column. While the ovens are cooling you can prepare a new oxidation column.
2. We have two types of quartz tubes, opaque and clear. It is easy to observe the packing and measure the amount of material added to a clear tube but more difficult to do the same on the opaque tubes. When using the opaque tubes the push rod and template will assist you in determining the amount of material in the tube.
3. Insert 3.5 to 4.0 cm of quartz wool into the bottom of the quartz reduction tube (Fig. P4). Place bottom of tube against the tray and use the push rod to tamp down the quartz wool (Fig. P4).
4. Using a funnel add approximately 6.0 to 6.5 cm of silvered cobalt (II, III) oxide to the quartz tube (Fig. P4). Gently tap the tube to compact the reagent. It is necessary to insure good packing of all reagents added to the quartz tube or separations will occur inside the tube when the column is heated to 1020°C. Use the push rod as a measuring device against the template. The total accumulation of both quartz wool and silvered cobalt (II, III) oxide should be 10 cm.
5. Add 1.0 cm of quartz wool to separate the silvered cobalt (II, III) oxide from the chromium (III) oxide.
6. Again using a funnel, add 11.0 cm of chromium (III) oxide to the quartz tube (Fig. P4). As you add the reagent to the tube gently tamp the tube to compact the reagent to avoid separation when the column is heated to 1020°C. Use the push rod to measure the amount of chromium (III) oxide added.
7. Add approximately 1.0 to 1.5 cm of quartz wool on top of the chromium (III) oxide for a total of 23.5 cm (Fig P5).
8. Prepare a quartz insert by placing 5 to 7 mm of quartz wool at the bottom of the tube. Slide the insert into the top of the oxidation column. The insert should extend past the top of the oxidation column by 1 to 2 mm. If the insert is flush or below the top of the oxidation column you will need to add an appropriate amount of quartz wool to the column (Fig P5).
9. Install a black sealing ring at the top with the flat portion down (Fig P6).
10. Clean the outside of the oxidation tube with a Kimwipe and 2-propanol and set the tube aside (Fig P6).

11. When the oven has cooled and the new oxidation column is ready you can begin removing the used oxidation column.
12. Remove the furnace cover to expose the bottom of the oxidation column (Fig. P7).
13. Remove both braces at the bottom of the oxidation and reduction columns (Fig. P8).
14. Completely loosen the fittings on the bottom of the reduction column (right) and the bottom of the oxidation column (left) and remove the coupling (Fig. P8).
15. Loosen the fittings connecting the carousel to the oxidation reactor column and helium input line. Move carousel to the side so the oxidation column can be removed without obstruction (Fig. P9).
16. Carefully remove the used oxidation column and install the new one (see caution note in Fig. P9).
17. Assemble in reverse order. Attach the carousel first and then install the bottom nut, washer, and sealing ring on the bottom of the oxidation column. Replace the coupling and secure both fittings (Fig. P8).
18. Install both braces (Fig. P8) and perform a leak check EA (Fig. P10).
 - a. Place a plug over Vent-M, release pressure on M helium regulator on EA (turn black/silver knob counterclockwise).
 - b. If there is a leak the pressure on the M helium gauge will decrease. If this occurs check for leaks. The most common source of leaks will be at the carousel connection to the reactor column, seals in water trap, or the carousel lid seal. Make sure to restore helium pressure to 90kPa before checking for leaks. Repeat 9 and 18b if necessary until leak is repaired.
 - c. If there is no leak, remove plug on Vent-M and restore helium pressure to 90kPa.
19. Turn up the oxidation oven to 1020°C and the reduction oven to 650°C (Fig. P3). When the ready light on the front panel illuminates the instrument is ready for use.
20. It is common after an oxidation column change that you will need to change the water trap on the EA because of the moisture released from the column reagents. See Section 3D for this procedure.



Figure P1: Material required to prepare oxidation column on the Carlo Erba NA1500 Series II elemental analyzer.



Figure P2: Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M on the left to Vent-R in the middle. The oxygen vent is on the right.



Figure P3: Turn down the oxidation oven from 1020°C to 320°C and the reduction oven from 650°C to 250°C. Allow the ovens to cool before removing the reduction column. You will have plenty of time to prepare a new column.



Figure P4: Preparing oxidation column. Insert 3.5 to 4.0 cm of quartz wool into the bottom of the quartz tube (upper left photo). Gently tamp the quartz wool with the push rod (upper middle photo). Then add 6.0 to 6.5 cm of silvered cobalt (II, III) oxide (upper right photo) for a combined total of 10 cm. Add 1 cm of quartz wool (bottom left photo) and again gently tamp using push rod (bottom middle photo). Next add 11 cm of chromium (III) oxide on top of the quartz wool.



Figure P5: Continued preparation of oxidation column. Insert 1.0 to 1.5 cm of quartz wool on top of the chromium (III) oxide (left photo). Prepare a quartz insert by placing 5 to 7 mm of quartz wool in the bottom of the insert (middle photo). Slide the insert into the top of the oxidation column (right photo). The top of the insert should extend approximately 1 to 2 mm above the top of the column. If it falls below add an appropriate amount of quartz wool to compensate.

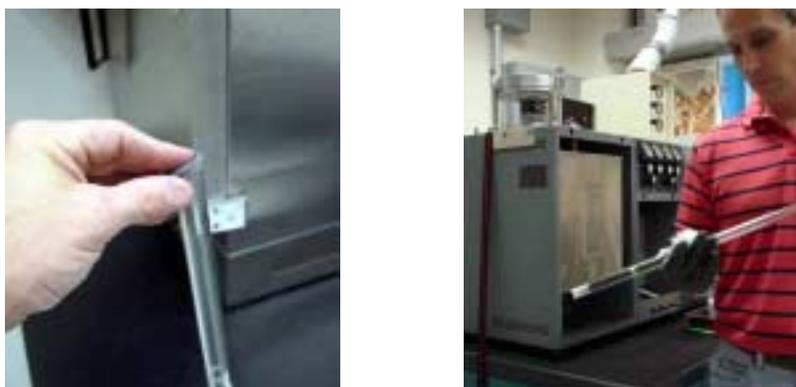


Figure P6: Install sealing ring on top of the reduction column (left photo). Clean the outside of the reduction column using a Kimwipe and 2-propanol.



Figure P7: Before you can service the columns you need to remove the oven cover to the elemental analyzer. To accomplish this slide the cover up until it stops then pull it toward you.

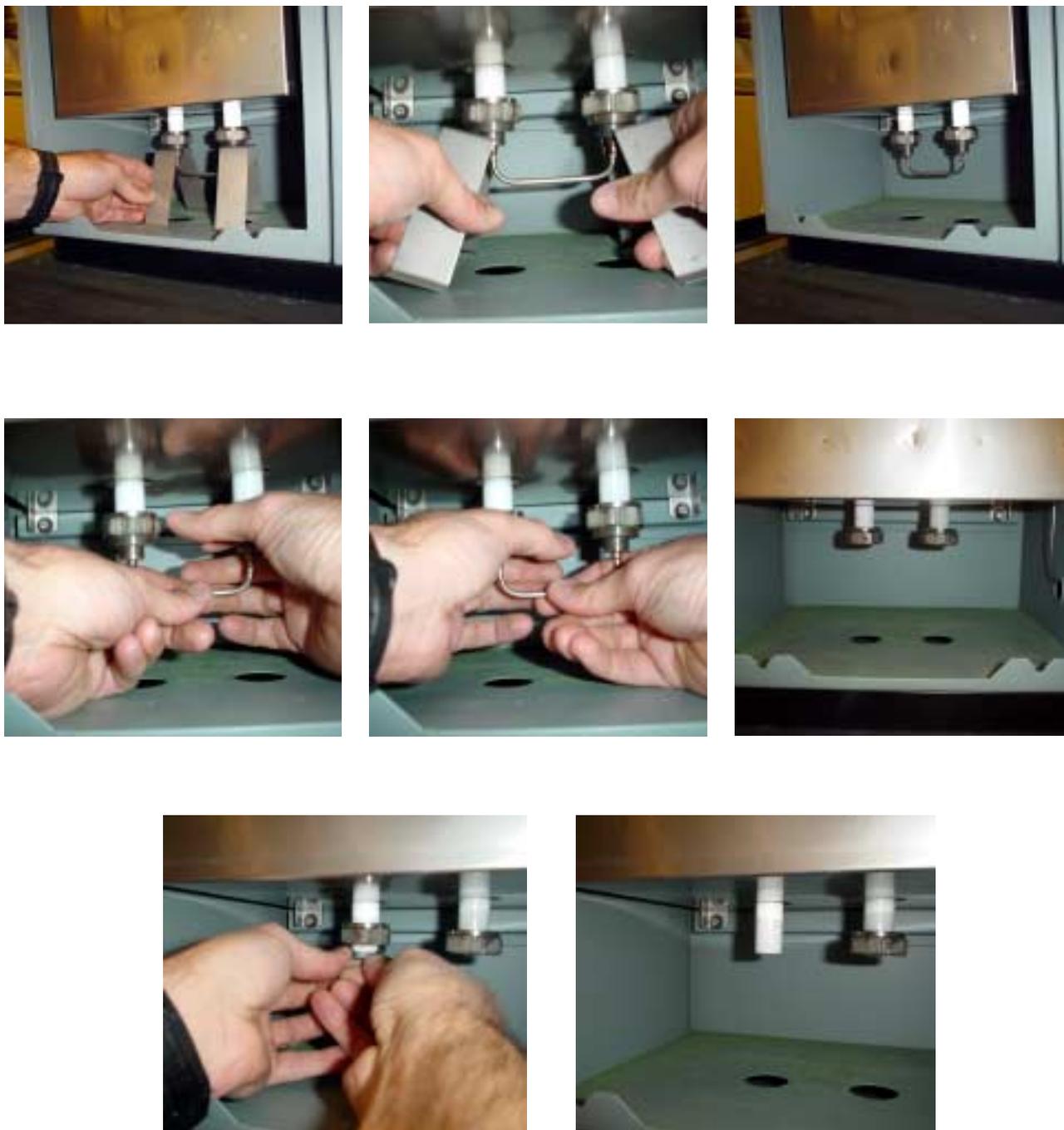


Figure P8: Disassemble the coupling joining the bottom of the oxidation and reduction columns (upper left photo). Remove both supports (upper middle photo) to expose the coupling (upper right photo). Completely loosen the fitting to the reduction column (middle left photo) and the fitting to the reduction column (center photo) and remove coupling (middle right photo). Remove the sealing ring, washer, and nut from the bottom of the oxidation column (bottom left photo) so that the column is free (bottom right photo).

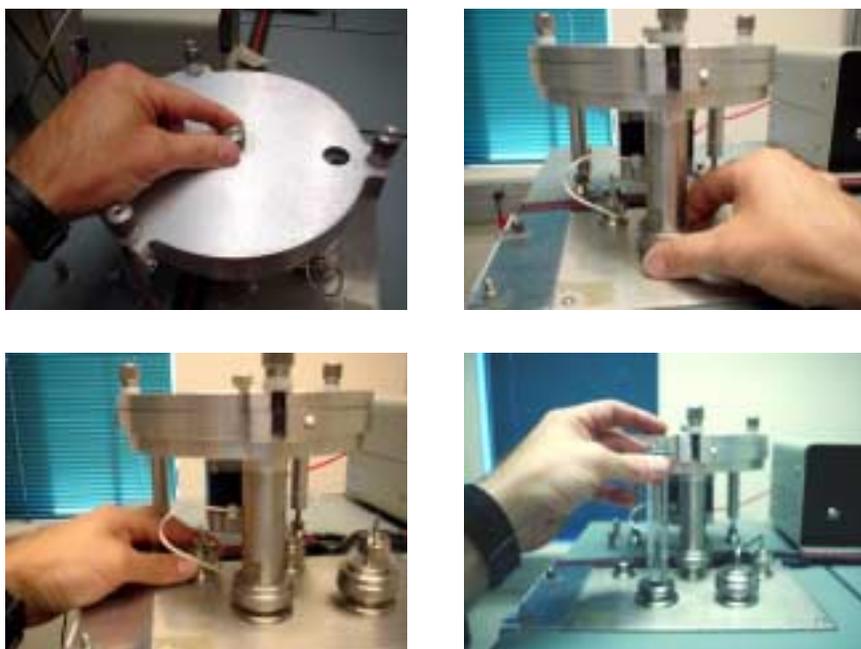
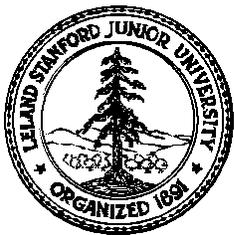


Figure P9: Removing the Costech Analytical zero blank carousel from EA reactor column. First open the purge valve on top (upper left photo) then loosen the connections to the oxidation column (upper right photo) and the helium line (lower left photo). Remove oxidation column (lower right). **Caution:** Handle with care, the oxidation column is still hot around 350°C to 400°C. Place upright in a beaker until cool.



Figure P10: Leak checking the Carlo Erba NA1500. Move stainless steel line from Vent-M to Vent-R (see Fig. 2) and place plug (left photo) on Vent-M. Turn down (counter clockwise rotation) the helium pressure by using the knob labeled M on the EA (right photo). Observe the M helium gauge for leaks. When finished restore helium pressure to 90kPa (clockwise rotation).



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Section 3D: Preparation of Elemental Analyzer Water (magnesium perchlorate) Trap

This procedure is designed to prepare a water (magnesium perchlorate or anhydrous) trap for the Carlo Erba NA1500 Series II elemental analyzer (EA). The entire procedure should be performed over a secondary container, such as a tray, to capture any spilled materials. Safety glasses, rubber gloves, particle mask and a lab coat/apron are required throughout the acid preparation.

Unlike the oxidation and the reduction columns on the EA the water trap needs to be changed on a regular basis (i.e., every 1 to 3 days or approximately 80 to 200 runs) depending on what type of samples are being analyzed. This can best be determined by monitoring consistency of the magnesium perchlorate in the clear glass trap tube. As the magnesium perchlorate becomes wet it begins to clump together. When the upper 1 cm shows obvious texture change then it is time to change the trap. You can also use the ISODAT software on the Finnigan Delta_{plus} mass spectrometer to monitor mass 18 on beam 2. This reading is normally below 3 V so if it is higher the trap needs to be changed. In order to keep the magnesium perchlorate granules dehydrated they are stored in a flask in a helium environment supplied by the purge on the elemental analyzer (Fig. P1). Make sure you replace the cap to the flask when you are finished. The purpose of this section is to serve as a guide, you should not attempt this unless you have been trained and have a good understanding on operating the EA and the mass spectrometer. Before you begin preparing the new water trap, you should first clear an area on the bench top, obtain and organize all of the materials and equipment listed below.

Materials: Costech Analytical grade magnesium perchlorate and quartz wool (Fig. P1).

Equipment: Forceps, funnel, spatula, and tray (Fig. P1).

I: Removing water trap

1. Switch the stainless steel line from Vent-M to Vent-R (Fig. P2).
2. Remove the magnesium perchlorate water trap. Hold the glass tube and loosen red caps on both ends of trap and remove. Teflon/silicon seals usually stick to the glass trap. Remove the seals and check for cracks. Replace the seals if necessary otherwise clean and use (Fig. 3).
3. Prepare water trap. Clean water trap tubes are located in the stainless steel oven. Use a funnel and put 5 to 7mm of quartz wool in one end of a clean trap tube and fill with magnesium perchlorate granules and gently tap trap on bench top to compact granules. Be sure to leave approximately 5 to 7mm of space in order to insert quartz wool into the open end (Fig 4).
4. Install the newly prepared water trap in the reverse of Step 2 (Fig. 3). Remember the Teflon face of the seal goes against the steel seat inside the red cap and the silicon face goes against the glass water trap tube.

II: Perform a leak check EA (Fig. P5).

1. Place a plug over Vent-M, release pressure on M helium regulator on EA (turn black/silver knob counterclockwise).
2. If there is a leak the pressure on the M helium gauge will decrease. If this occurs check for leaks. The most common source of leaks will be at the carousel connection to the reactor column, seals in water trap, or the carousel lid seal. Make sure to restore helium pressure to 90kPa before checking for leaks. Repeat 8a and 8b if necessary until leak is repaired.
3. If there is no leak, remove plug on Vent-M and restore helium pressure to 90kPa.

III: Cleaning water trap.

1. Remove quartz wool from on end of the trap to expose the magnesium perchlorate (Fig. P6).
2. Use a spatula to loosen the magnesium perchlorate and dispose in the labeled waste container (Fig. P6).
3. When all of the magnesium perchlorate has been emptied remove the remaining quartz wool and wash the trap with deionized water and place in oven at 60°C.
4. Secure lid on waste container.



Figure P1: Helium line feeding magnesium perchlorate storage flask (left photo). Material required to prepare magnesium perchlorate water trap on the Carlo Erba NA1500 Series II elemental analyzer (right photo).



Figure P2: Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M on the left to Vent-R in the middle. The oxygen vent is on the right.



Figure P3: Checking and changing the magnesium perchlorate water trap on the Carlo Erba NA1500. Observe upper left portion of trap to see if magnesium perchlorate is sticking or degrading. To remove the water trap loosen the red caps on both ends of the glass trap (left and middle photo). Remove Teflon/silicon seals from both ends and check for cracking (right photo). Replace if necessary or reuse seals. Make sure silicon portion of seal seats against the glass trap and the Teflon against the stainless steel flange.



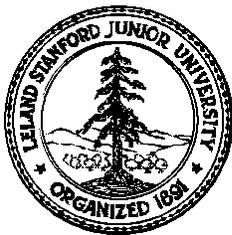
Figure P4: Preparing new water trap. Insert 5 to 7 mm of quartz wool into the bottom of the water trap tube (left photo). Use a funnel to add magnesium perchlorate to the tube (middle photo), make sure you gently tap the tube to slightly compact the magnesium perchlorate to avoid separation. Make sure you leave a 5 to 7 mm space in the trap tube to insert quartz wool into the open end (right photo).



Figure P5: Leak checking the Carlo Erba NA1500. Move stainless steel line from Vent-M to Vent-R (see Fig. 2) and place plug (left photo) on Vent-M. Turn down (counter clockwise rotation) the helium pressure by using the knob labeled M on the EA (right photo). Observe the M helium gauge for leaks. When finished restore helium pressure to 90kPa (clockwise rotation).



Figure P6: Cleaning water trap. Remove quartz wool from one end (left photo). Use a spatula to loosen the magnesium perchlorate in the trap tube (middle photo). Dispose of used magnesium perchlorate into labeled waste container (right photo). Remove remaining quartz wool from trap tube, then was the trap tube in deionized water.



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Section 3E: Changing Elemental Analyzer quartz insert

This procedure is designed to illustrate how to change the quartz insert in the oxidation column on the Carlo Erba NA1500 Series II elemental analyzer (EA). Unlike the oxidation and reduction columns and water trap on the EA the quartz insert needs to be changed after each run sequence (i.e., every 31 to 49 samples) depending on what type of samples are being analyzed and the drum used.

When a sample is dropped into the reactor or oxidation column on the EA the sample contained in either a tin or silver capsule is combusted and subsequently turned into an ash deposit. Obviously samples combusted in tin and having a high organic content will ash better than samples with high clay content in silver capsules or glass fiber filters in tin capsules. The total number of samples that can be analyzed in a run sequence depends on how well the sample combusts in the oxidation column. Table 1 shows examples of sample runs where the total number of runs includes conditioners, blanks, standards, and samples. Since our lab uses the Costech Analytical zero blank carousel we have adopted the following protocol. This carousel can use either a 32 or 50 hole drum where the sample yield is 31 and 49, respectively because one of the holes in each drum is oriented over the top of the oxidation column. In order to extend the run and add more samples to the carousel the run must be stopped, carousel opened, loaded, and purged. It only takes a few extra minutes to add an insert change to this process thereby reducing the risk of bad data due to excess ash accumulation. To set up and run a 31 sample sequence takes approximately 5.5 to 6 hours and a 49 sample run takes 7.5 to 8 hours. Before you begin preparing the new water trap, you should first clear an area on the bench top, obtain and organize all of the materials and equipment listed below.

Table 1: Number of conditioners, blanks, standards, and samples that can be run on one quartz insert using the Costech Analytical zero blank carousel.

Material	Weight (mg)	Total number of runs
Soils	20 - 35 mg	31
Sediments	20 - 35 mg	31
GF/C filter	N/A	31
Plants/leaves	2 - 5 mg	49
Animal parts	1 - 5 mg	49
High organic material	0.1 - 1 mg	49

Materials: Costech Analytical quartz insert and quartz wool (Fig. P3).

Equipment: Sharpie felt pen or forceps (Fig. P2).

I: Removing the quartz insert

1. Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M to Vent-R (Fig. P1).
2. Remove quartz insert from EA reactor/oxidation column:
 - a. Loosen purge valve on top of carousel (Fig. P2).
 - b. Next loosen the fittings connecting the carousel to the reactor column and helium input line. Move carousel to the side so the insert can be removed without obstruction (Fig. P2).
 - c. Carefully remove insert from reactor. Care should be taken when handling the insert because it is at 1020°C. Place hot insert into waste beaker (Fig. P3).
3. Install new quartz insert into EA reactor column:
 - a. Put approximately 5 to 7 mm of quartz wool into the bottom of the insert (Fig. P3).

- b. Carefully slide the quartz insert into reactor column until it stops (do not push or force insert, Fig. 3). The insert should stop approximately 5mm above top of reactor column). If the insert falls below the top of the reactor column add an appropriate amount of quartz wool to the reactor column (not the insert) then slide the insert in again.
- c. With the insert in the proper position, gently push the insert down until it is flush with the top of the reactor column (do not force).
- d. Reposition the carousel onto the reactor column and secure both connections at the reactor column and the helium carrier line (Fig. P4).
- e. Let the carousel to purge for approximately 2 minutes to remove the atmosphere from the carousel headspace. Then close the purge valve.
- f. Allow about 7 minutes for the air to purge the EA plumbing. This can be monitored using the meter on the front panel of the EA.
- g. When the meter reaches baseline (< 750) you can perform a leak check.

II: Perform a leak check EA (Fig. P5).

1. Place a plug over Vent-M, release pressure on M helium regulator on EA (turn black/silver knob counterclockwise).
2. If there is a leak the pressure on the M helium gauge will decrease. If this occurs check for leaks. The most common source of leaks will be at the carousel connection to the reactor/oxidation column or the carousel lid seal. Make sure to restore helium pressure to 90kPa before checking for leaks. Repeat 1 and 2 if necessary until leak is repaired.
3. If there is no leak, remove plug on Vent-M and restore helium pressure to 90kPa.



Figure P1: Switch stainless steel line exiting the elemental analyzer (EA) from Vent-M on the left to Vent-R in the middle. The oxygen vent is on the right.

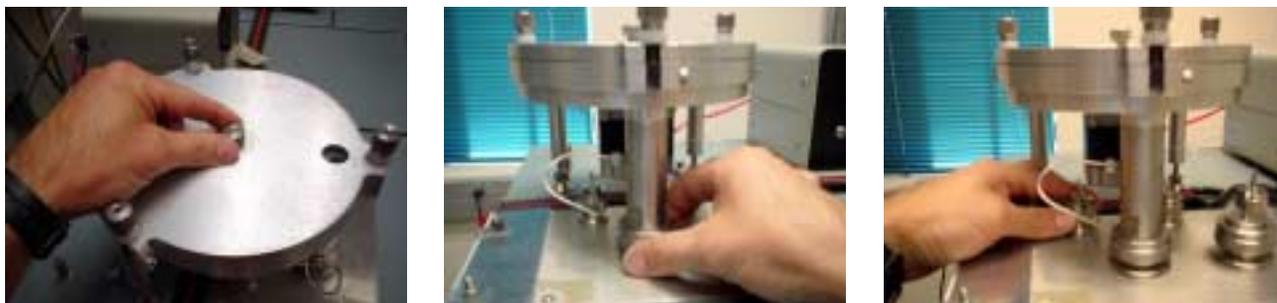


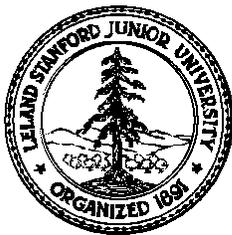
Figure P2: Removing the Costech Analytical zero blank carousel from EA reactor column. First open the purge valve on top (left photo) then loosen the connections to the reactor column (middle photo) and the helium line (right photo). Then move carousel to the side to allow removal of the quartz insert.



Figure P3: Removing quartz insert from EA reactor column. A marker pen works great at removing the insert (left photo). Once the insert is removed enough grab the top with your fingers, remove completely and place into the waste beaker (middle photo). Note the location of the carousel. In this position there is no obstruction when removing the insert. Place approximately 7 mm of quartz wool in the bottom of the insert and slide it into the reactor column (right photo).



Figure P4: Leak checking the Carlo Erba NA1500. Move stainless steel line from Vent-M to Vent-R (see Fig. 2) and place plug (left photo) on Vent-M. Turn down (counter clockwise rotation) the helium pressure by using the knob labeled M on the EA (right photo). Observe the M helium gauge for leaks. When finished restore helium pressure to 90kPa (clockwise rotation).



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Section 6A: Biogenic Silica Analysis Procedure

I) Introduction

This procedure is designed to give weight percent biogenic silica using a small amount of sample material. A total of 42 analyses (40 unknowns, 1 blank, 1 standard) can be run using this procedure. This procedure uses a 0.1M NaOH solution at 85°C to leach the silica from the sample. This procedure has been modified from Strickland and Parson (1972).

Materials: Sodium hydroxide (NaOH), Sodium fluorosilicate (Na_2SiF_6), analytical grade reagent Oxalic acid dihydrate $[(\text{COOH})_2 \cdot 2\text{H}_2\text{O}]$, Ammonium paramolybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$, Hydrochloric acid (HCl), Sulfuric acid (H_2SO_4), Anhydrous sodium sulfite (Na_2SO_3), Metol P-methylaminophenol sulfate, Nanopure water (silica free).

Equipment: Spectrophotometer @ 812nm, constant temperature bath, centrifuge, adjustable pipette (dispense 2 and 3 ml) with disposable tips, micro pipettes (250 & 500 μl) with disposable tips, numbered Nalgene 12 ml vials with caps, numbered Nalgene 50 ml centrifuge tubes, 1000 ml volumetric flask, 4-500 ml volumetric flasks, 50 ml Nalgene volumetric flasks, 1200 ml stainless steel beaker, 1 liter Nalgene bottle, safety glasses, gloves, and lab coat.

II) Reagents

- Sodium hydroxide solution:** make a 0.1M sodium hydroxide (NaOH) solution by dissolving 16.0 g NaOH in 4 liter of Nanopure water in a 4 liter Nalgene bottle. Let sit or mix until the NaOH is completely dissolved.
- Molybdate reagent:** In a fume hood, dissolve 8.0 g ammonium paramolybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ in approximately 600 ml of Nanopure water using a 1000 ml volumetric flask. Add 24 ml concentrated hydrochloric acid (12N/37% HCl) to the ammonium paramolybdate solution, mix, and then bring the total to 1000 ml using Nanopure. Store in a brown polyethylene bottle out of direct sunlight. The reagent is stable for many months, but should be discarded if any white precipitate forms (enough for 2 runs).
- Metol-sulfite solution:** In a fume hood, dissolve 12.0 g anhydrous sodium sulfite (Na_2SO_3), in approximately 600 ml Nanopure water using a 1000 ml volumetric flask and. Add 20.0 g metol P-methylaminophenol sulfate to the same volumetric flask, mix and fill to 1000 ml. When the metol has dissolved, filter the solution through a No. 1 Whatman filter and then store in a clean dark glass bottle. This solution may deteriorate quite rapidly and should be made fresh every month.
- Oxalic acid solution:** In a fume hood, prepare a saturated oxalic acid solution by adding 60.0 g of oxalic acid dihydrate $[(\text{COOH})_2 \cdot 2\text{H}_2\text{O}]$ with 1000 ml Nanopure water in a 1000 ml volumetric flask. Let stand overnight. Decant solution from crystals into a glass storage bottle. The solution is stable indefinitely.
- Sulfuric acid solution (18N 50%):** In a fume hood, using a 1000 ml volumetric flask, pour 300 ml concentrated sulfuric acid (36N/98% H_2SO_4), into a 1000 ml volumetric flask. Cool to room temperature and bring volume to 1000 ml. Store in a 1 liter Nalgene bottle.

6. **Reducing solution:** This solution is mixed for each set of samples. In a fume hood, use a graduated cylinder to measure out equal amounts of Metol-sulfite solution, Oxalic acid solution, and Sulfuric acid. The amount of reducing solution required depends on the number of samples, blanks, standards and number of hours sampled. At the end of your sampling you will have to add 3.0 ml of reducing solution to each vial. For example: You have 17 samples, 1 blank and 9 standards, and you sample each hour for a total of 5 hours. You will generate 5 vials per sample or $(18 \text{ samples} * 5 \text{ vial/sample} * 3 \text{ ml}) + (9 \text{ standards} * 3 \text{ ml/standard}) = 297 \text{ ml}$ of reducing solution or 300 ml total reducing solution. As a precaution, you should make about 360 ml which is divisible by 3 (120ml/ea reagent).
7. **Primary silica standard:** dissolve 0.5642 g in a Nalgene 1 liter volumetric flask fill to 1 liter with Nanopure water. Dissolution is slow, so allow at least 30 minutes. This cannot be rushed. The concentration of this standard is 3000 $\mu\text{moles Si/liter}$. Store in a 500 ml Nalgene bottle. Standard is stable indefinitely.

a. **Dilution from primary standard:** when making a dilution, use Nanopure water and store in Nalgene bottles. Using a 50 ml Nalgene volumetric flask, add the following amounts of primary silica standard and then bring the volume to 50 ml total.

30 $\mu\text{M Si}$ - 0.5 ml of primary Si std.	480 $\mu\text{M Si}$ - 8.0 ml of primary Si std.
60 $\mu\text{M Si}$ - 1.0 ml of primary Si std.	600 $\mu\text{M Si}$ - 10.0 ml of primary Si std.
120 $\mu\text{M Si}$ - 2.0 ml of primary Si std.	900 $\mu\text{M Si}$ - 15.0 ml of primary Si std.
240 $\mu\text{M Si}$ - 4.0 ml of primary Si std.	1200 $\mu\text{M Si}$ - 20.0 ml of primary Si std.
360 $\mu\text{M Si}$ - 6.0 ml of primary Si std.	

III) Procedure

A. Sample and instrument preparation:

1. Dry all samples in an oven at 60°C. Use the Sartorius micro balance (Fig. 1) to weigh out sediments using a micro balance and then place material in numbered centrifuge tubes (Fig. 2). Remember to keep good records. The amount of sample required depends on the amount of anticipated silica content. A good starting point is between 8 - 15 mg. for samples between 20% - 50% or 15 - 20 mg for samples in the range of 5% - 20%.



Figure 1: Sartorius micro balance operation. Turn microbalance on by pressing the black button on the left. To tare the micro balance press the red button on the right.



Figure 2: Weighing out samples for biogenic silica analyses and placing them into centrifuge tubes.

2. Turn on Hitachi U-2001 spectrophotometer to allow it to stabilize. Zero instrument using Nanopure water. See Part IV in this section for Hitachi spectrophotometer instructions.
3. Fill Blue-M water bath with deionized water (DI) and turn on water bath (Fig 3). The water bath should be preset to 85°C, however, insert thermometer and adjust if necessary.



Figure 3: To operate Blue-M water bath, turn on using toggle switch as shown on the left. If necessary adjust the temperature setting using the large black knob as illustrated on the right.

4. Fill a 1 liter Nalgene bottle with 0.1M NaOH and insert the 50 ml Brinkmann Dispensette into bottle and set for 40 ml.

- Using the Brinkmann Dispensette add 40 ml to each labeled centrifuge tube, cap tightly, shake, insert into 85°C water bath, record time (Fig. 4). All of the tubes must be shaken every 30 minutes (Fig. 4)

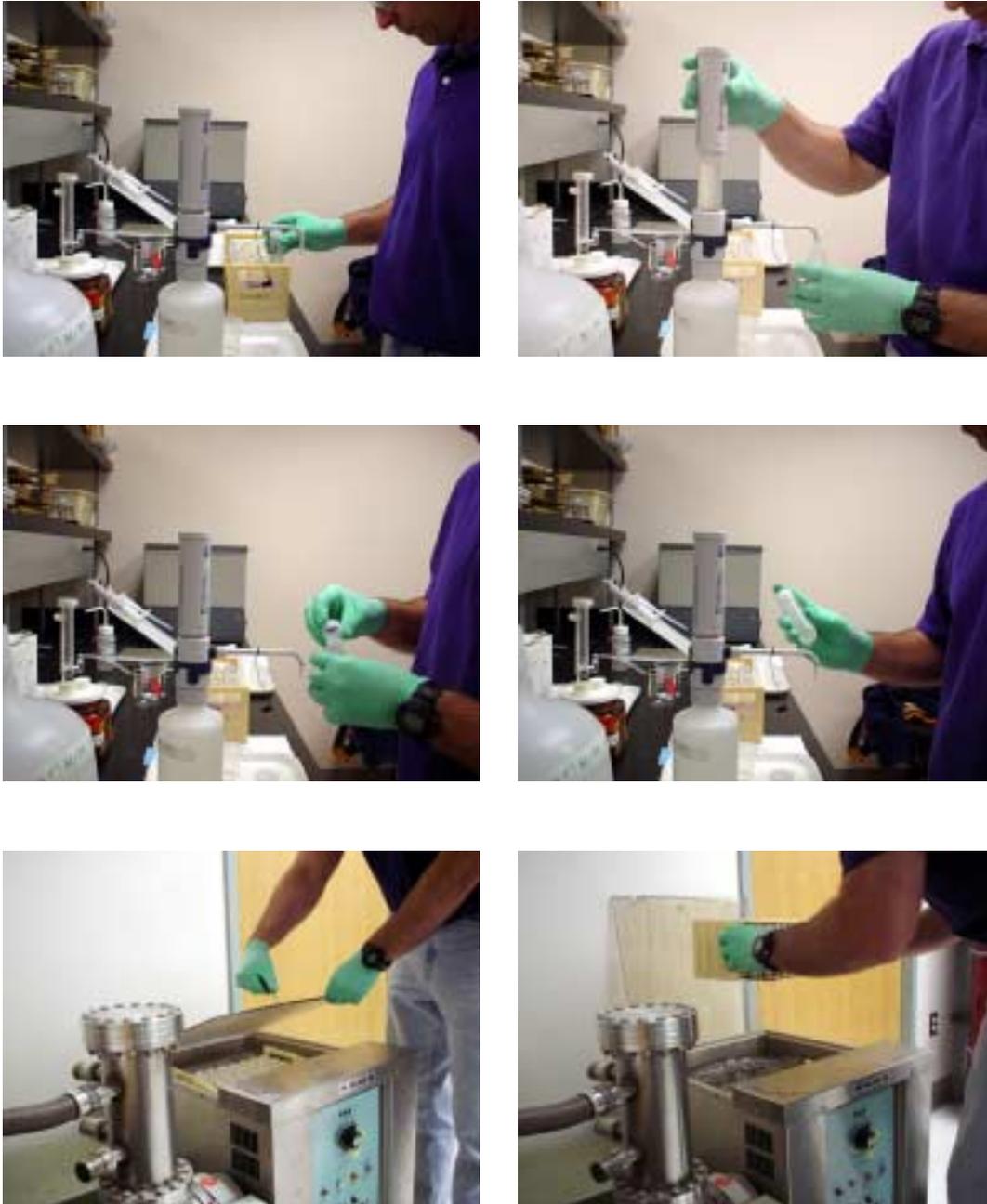


Figure 4: Prepare samples for biogenic silica analyses by adding 40 ml of NaOH to each centrifuge tube, cap, shake and place into the water bath at 85°C. You will need to shake the samples every 30 minutes.

B. Sampling:

Depending on the sample material being analyzed (sediment trap or sediments) your sampling time may vary. For example, when analyzing sediment trap samples we only sample the 2 and 3 hours. all other sediments we sample hours 1 through 5. The amount of solution taken on the selected hour can vary from 0.1 to 0.5 ml, however, we usually take 0.25 ml. If less than 0.5 ml is taken you want to add enough Nanopure to bring the total volume to 0.5 ml.

1. For simplicity, lets assume you take a sample every hour for five hours. This means you will need five 12 ml vials for each sample you plan to analyze (remember to always include the blank). All vials should be numbered and records kept to prevent confusion (Fig. 5).



Figure 5: Place numbered 12 ml standard and sample vials on tray. The tray is required as secondary containment in the event of a spill. It will also facilitate carrying the vials to the spectrophotometer.

2. On each hour remove the first eight centrifuge tubes and place into the centrifuge and spin for approximately 3 minutes (Fig. 6). See Figure 7 for centrifuge operation procedure. Remove tubes from centrifuge and place into a centrifuge tube holder. Place the second eight sample tube in the centrifuge and spin for 3 minutes. While the second set is spinning, take 0.25 ml (250 μ l) of sample from each tube using a 250 μ l pipette and place it in the appropriate vial (Fig. 8). Add 250 μ l of Nanopure for a total of 0.5 ml (500 μ l) then rinse pipette tip with Nanopure water (Fig. 9). Cap each tube tightly, shake, and place it back into the bath. This sample routine takes approximately 3 to 5 minutes for eight tubes. Take the second set of eight tubes from centrifuge and replace with the next set, let spin for 3 minutes, while spinning sample the second set. Continue until all tubes are sampled for the hour.



Figure 6: At sample time remove 8 centrifuge tubes from water bath and place them into the centrifuge. Lift the lid until it locks into place. To close, release the lid lock slide the bar back as illustrated in the right photo.



Figure 7: To operate the centrifuge flip the toggle switch up as shown in the upper right photo. Off is in the middle and the brake is in the down position. Dial the rotation speed to 10 using the black knob on the right of the front panel then set the time to 3 minutes using the black knob on the left of the front panel. This will start the centrifuge. At the end of this time the centrifuge will turn off automatically. You can apply the brake by flipping the toggle switch down. Allow the rotor inside the centrifuge to spin down to a stop before opening the lid and removing samples.



Figure 8: To sample, remove cap and pipette out 250 μ l from each centrifuge tube and place into a labeled 12 ml vial.



Figure 9: After taking 250 μl of sample from the centrifuge tube add 250 μl of Nanopure water to the 12 ml vial then rinse the pipette tip with Nanopure water before taking the next sample.

3. After the last sample has been taken for the hour and all tubes are back in the bath. Use the Lab Industries Repipet, set and add 4.5 ml of Nanopure water to each of the vials to bring the total volume to 5.0 ml (Fig. 10).



Figure 10: After taking 250 μl of sample from the centrifuge tube and adding the 250 μl of Nanopure water to the 12 ml vial (total volume of 0.5 ml) use the repipette to dispense 4.5 ml of Nanopure water to each vial.

4. After the first hour (or first sampling), prepare the standards. Using the concentrations prepared earlier in the outline described above in Part II, Number 7 of the Reagents section, add 0.5 ml (500 μl) of each concentration to a vial using a 500 μl pipette. Use the Lab Industries Repipet, set and add 4.5 ml of Nanopure water to each of the vials to bring the total volume to 5.0 ml.

C. Adding reagents:

Once all of the samples have been taken, you will be adding 2 ml of Molybdate reagent and 3 ml of Reducing solution to each of the sample, blank, and standard vials. Timing is important when adding these reagents. You will begin by adding the Molybdate reagent then the Reducing solution. However, you have to wait a minimum of 10 minutes to a maximum of 30 minutes before you can add the Reducing solution. So if it takes 20 minutes to go from vial 1 to vial ### at about 10 seconds per vial, when you finish vial ### you can begin adding the Reducing solution to vial 1 because it took more than 10 minutes. However, you must now take the same amount of time to add the Reducing solution to each vial.

1. Use the Oxford adjustable pipette and set to 2 ml. Noting the time, add 2 ml of Molybdate reagent (Part II, Number 2 in the Reagents section) to each of the sample, blank, and standard vials (Fig. 11). Remember you have between 10 and 30 minutes from the time you start adding Molybdate reagent from the first to last vial before adding the Reducing solution.



Figure 11: Noting the time add 2 ml of Molybdate reagent to each 12 ml vial. Then wait a minimum of 10 min but no more than 30 min before adding the reducing solution.

2. Use the Oxford adjustable pipette and set to 3 ml. Noting the time, add 3 ml of Reducing solution (Part II, Number 6 in the Reagents section) to each of the sample, blank, and standard vials. Remember you have add the Reducing solution using the same timing as the Molybdate reagent.



Figure 12: Noting the time add 3 ml of reducing solution to each 12 ml vial using the same timing as the Molybdate solution.

3. Cap all vials and let stand for 3 hours, no more than 5 hours before measuring their absorbance on the Hitachi spectrophotometer.



Figure 12: When finished adding the reducing solution cap all vials and let stand a min of 3 hours but not more than 5 hours. Standards are located in the front from 0 to 1200 μM (left to right).

IV) Hitachi U-2001 operational procedure

The Hitachi U-2001 (Fig. 1) is a programmable spectrophotometer that uses a small volume sipper attachment for individual sample analysis. This instrument has already been programmed for the analysis of biogenic silica. You should also turn the instrument on approximately 20 to 30 minutes prior to sample analysis. The instrument needs time to stabilize. Please treat the instrument with care and clean up your work area when finished.



Figure 1: Hitachi U-2001 Spectrophotometer

1. Remove instrument dust cover.
2. Turn the spectrophotometer on. Switch is located on the front lower left. The instrument will make some noise as it goes through several self check sequences that can be observed on the display. When complete, the main menu will appear on the instrument display with a cursor flashing at the <SELECT OPTION> at the bottom left. Remember to turn the instrument on 20 to 30 minutes prior to analyzing samples.
 - a. At the 'SELECT OPTION' prompt, type 6 <ENTER>. This is the 'TEST MENU' routine.
 - b. At the 'SELECT OPTION' prompt, type 1 <ENTER>. This is the 'LOAD TEST' routine.
 - c. At the 'SELECT OPTION' prompt, type 20 <ENTER>. 'DUNBAR' This is the preprogrammed routing for running biogenic silica. The instrument will automatically tune the correct wavelength of 812nm.
3. Fill the reference cuvet 3/4 full with Nanopure water. Slide the cover above the sipper back and insert cuvet into reference cell chamber (Fig. 2). The reference cuvet should be oriented so that the clear portions of the cuvet facing the sides (i.e., frosted sides front to back).
4. Pull back the pump tube tensioning plate until you hear a click and the release button pops up. Then slide the cover above the sipper closed (Fig. 3).
5. Place the spectrophotometer drain line into a waste container and release the clamp.
6. Insert the sipper tube into a beaker containing about 50 ml of Nanopure water. Flush about 10 ml to 20 ml of Nanopure water into the cell using the gray 'WASH' button (Fig P4) on the front of the sipper until all of the lines are filled and there are no bubbles.



Figure 2: Installing the Reference cell into the Hitachi U-2001 Spectrophotometer. Push back the lid to the auto sip device (left photo) then locate the reference cell port in the back right corner (middle photo) and gently insert the reference cell into the port (right photo) with the clear portion of the cell facing both sides of the instrument.

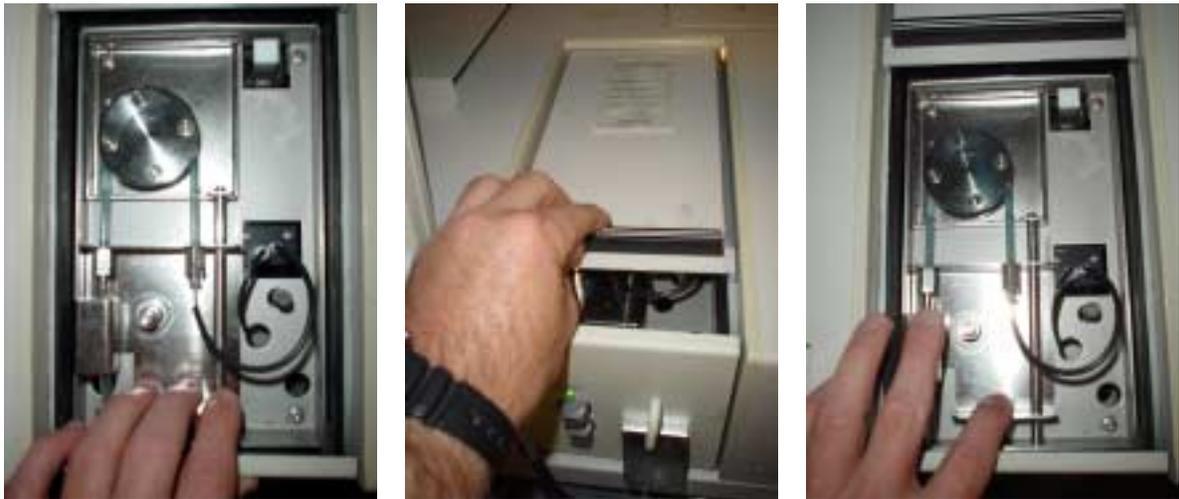


Figure 3: Engage/disengage tension plate on the Hitachi U-2001 Spectrophotometer auto sip device. Pull back the pump tube tensioning plate until you hear a click (left photo) and the release button pops up. Then slide the cover above the sipper closed (middle photo). When finished press the release button (right photo) and then close sipper lid (middle photo).

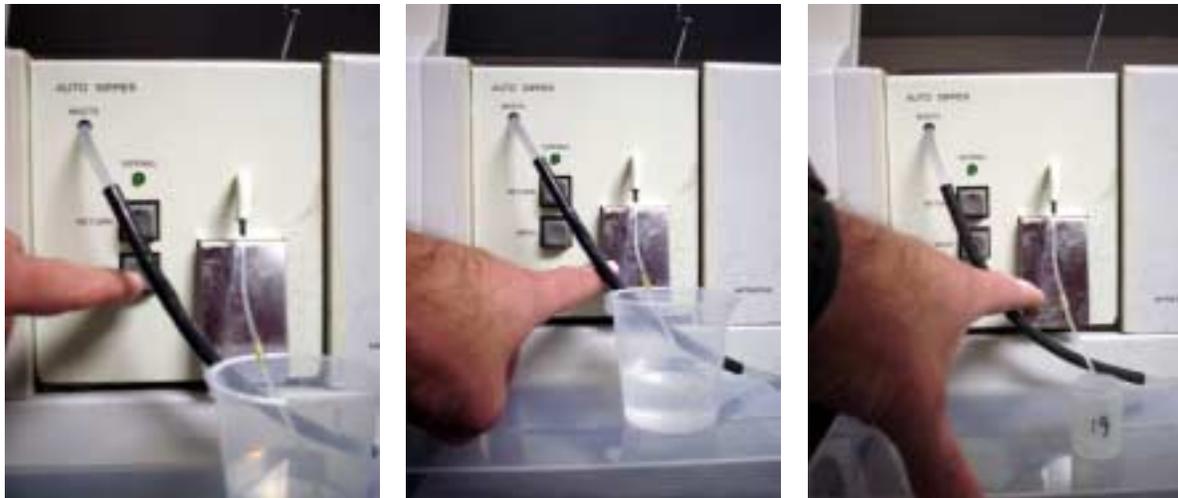


Figure 4: Operating the Hitachi U-2001 Spectrophotometer auto sip device. Fill sipper cell, using the WASH button (left photo), with Nanopure water prior to performing an AUTO ZERO. Use the stainless steel sip actuator (middle photo) to sample Nanopure water for AUTO ZERO. When ready use the sample sip actuator to run standards, unknowns, and blanks (right photo).

7. Run 3 to 5 sample cycles through the cell by pressing the large stainless steel actuator on the front of the sipper. After each run the instrument will display the result on the screen. When the results of these runs are consistent press the AUTO ZERO on the keypad (Fig P5). Sample the Nanopure water 3 to 5 time again to make sure the instrument is stable at 0.000 ± 0.001 . The instrument is now ready for standards.

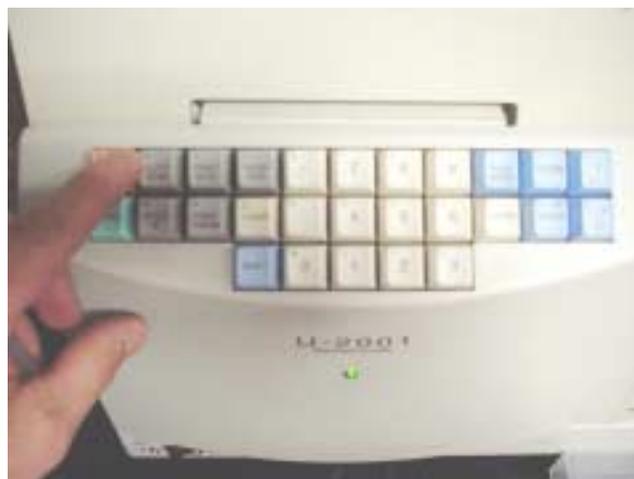


Figure 5: AUTO ZERO the Hitachi U-2001 Spectrophotometer. Fill sipper cell, using the WASH button, with Nanopure water prior to performing an AUTO ZERO. Use the stainless steel sip actuator to sample Nanopure water for AUTO ZERO. When the results are consistent, Press the AUTO ZERO key. Then repeat 3 to 5 more times to make sure the value is 0.000 ± 0.001 .

8. Standards should always be performed first to insure that the instrument and biogenic silica chemistry are sound. Once verified the blanks and unknown samples can be done.

9. Insert the sipper tube into the standard/sample vial and press the stainless steel actuator (Fig P4). Keep in mind the first reading may not be valid but will serve mostly to flush out the previous standard/sample. Subsequent readings, up to 3 in a 10 ml volume, should be accurate and reproducible to ± 0.005 . Record the readings after each sampling. Also insure the sipper tube remains in the liquid so that bubbles are not introduced into the sipper cell. Finally, depending on the color difference between samples more sips may be required before the results are reproducible.
10. When all samples have been analyzed, intermittently for large batches of samples, run Nanopure water to check for instrument drift. Record the readings and continue or go to the next step.
11. Place the sipper tube into a beaker containing approximately 50 ml of 0.1M NaOH. Flush the sipper cell by pressing the gray 'WASH' button on the front of the sipper (Fig 4). Next flush approximately 100 ml of Nanopure water through the system in the same manner.
12. Replace the clamp to the waste line
13. Slide the sipper cover open and release the tension on the pump tube by pulling back the tension plate and pressing the release button (Fig 3).
14. Remove the reference cuvet, drain and dry. Close the sipper cover.
15. Turn power off, replace dust cover.
16. Remove waste and dispose of properly.

V) Data reduction

When you are finished analyzing all of your standards, blanks, and unknowns you will need to enter the results into the OPAL Program (Vbasic Macro) to obtain a standards curve and weight percent values for the unknown samples. A simple linear regression is used to calculate the slope and intercept on the standard values entered into the program. The weight percent biogenic silica results are calculated using the following equation:

$$\text{Wt. \% SiO}_2 = ([\text{SiO}_2]) * (0.5 \text{ ml/aliquot}) * (\text{volume}) * (60.1 \text{ g Si/mole}) * (0.0001) / (\text{Sample wt.})$$

Where: $[\text{SiO}_2] = ((\text{absorbance} - y \text{ intercept}) / \text{slope})$

absorbance = reading from spectrophotometer.

aliquot = amount of sample taken from centrifuge tube in ml.

sample wt = weight of sample in mg.

slope = calculated from standards curve.

volume = amount of NaOH solution added to centrifuge tube in ml.

y intercept = calculated from standards curve.

0.0001 = $(1\text{E}-06 \text{ g}/\mu\text{g} * 100\%)$

60.1 g Si/mole = amount of Si in one mole of SiO_2 .

VI) Quick reference guide

1. Day prior to analyzing samples

- a. Dry samples in an oven at 60°C. Weigh out samples using micro balance and place into numbered centrifuge tubes. Leave in oven uncapped at 60°C to keep dry.

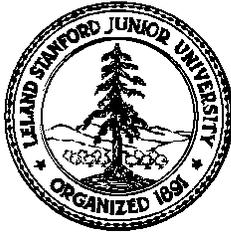
- b. Fill water bath with water and heat to 85°C. It is best to turn the water bath on the night before running to make sure it is up to temperature.

2. Run preparation.

- a. Carefully pipette 40 ml of 0.1M NaOH into each centrifuge tube, cap tightly, shake and insert into the water bath set at 85°C. Record time. All tubes must be shaken every 30 minutes.
- b. Depending on sampling time (i.e., each hour for five hours, 2nd and 3rd hour, etc.) remove 8 centrifuge tubes and place into the centrifuge and spin for approximately 3 minutes. Take 250 µl of sample from each tube using a pipette and place it into a labeled vial. Rinse the pipette tip with Nanopure water then add 250 µl of Nanopure water to the sample for a total of 500 µl (0.5 ml). Cap centrifuge tube tightly and place it back into the rack. Continue until all tubes are sampled then shake all tubes and return them to the water bath. Continue as needed until the sampling interval is completed.
- c. Add 500 µl of each silica standard to a labeled vial. This is only done after the first sampling.
- d. Use the Repipet to introduce 4.5 ml of Nanopure water into each standard and sample vial. All vials should now have 5.0 ml of liquid, including either 250 µl of sample or 500 µl of standard.
- e. Noting the time pipette in 2 ml of Molybdate reagent to each sample, blank, and standard. After no less than 10 minutes and no more than 30 minutes go to the next step.
- f. Noting the time pipette in 3 ml of Reducing solution to each sample, blank, and standard. Remember to need to use the same timing as the Molybdate reagent.
- g. Cap all vials and let stand for 3 hours, no more than 5 hours before measuring their absorbance on the spectrophotometer at 812 nm.

VII) References:

- DeMaster, D.J., 1981. The supply and accumulation of Silica in marine sediments, *Geochim Cosmochim. Acta*, 45, 1715-1732.
- Mortlock, R.A. and P.N. Froelich, 1989. A simple method for the rapid determination of biogenic opal in pelagic marine sediments, *Deep-Sea Research*, 36, 1415-1426.
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Stanford University Stable Isotope Laboratory
On-line Manual
(Prepared by David A. Mucciarone)

Section 6C: Sediment Trap Split Procedure

I) Introduction

This procedure is designed to split sediment trap samples (usually 500ml) collected in the field into working splits (sum of the splits totaling half of the sample) and an archive split (remaining half of the sample). Depending on the accumulation of material in the sample cup this split ratio can be modified to accommodate sample needs.

Safety Materials: Gloves, safety glasses, lab coat or apron.

Caution: Sediment trap samples contain about 5% formalin. Therefore, this procedure should be carried out in a fume hood.

Equipment: Plankton splitter, squeeze bottles (2-3), Nalgene bottles 125 mL and 250 mL sizes, forceps, Nuclepore or Milipore polycarbonate membrane filters (0.45 μ m x 47mm) , Plastic media dishes (60 x 15mm), Small trays (2), 47 mm filtering rig and vacuum line, 2ml cryogenic vials, Pipette and tips, 50 mL centrifuge tubes.

II) Preparation

1. Weigh out 4 Nuclepore filters for each sediment trap sample to be processed. Place each in a small plastic media dish with the weight clearly labeled on the lid.
2. On the log sheet (Fig. 1), record the name of the sample, your progress through out this procedure, any appropriate remarks, and to keep track of splitting at the end of the procedure.

III) Procedure

1. Allow the sediment trap container to settle at least over night. If possible longer is better. This will allow the particulate matter in the container to settle and thereby maximizing the clarity of the liquid fraction (supernatant).
2. Open the sediment trap sample container (usually 500ml) and remove 2ml supernatant from the top using a pipette. Fill 2ml cryogenic vial (or cryovial) with supernatant, label vial and refrigerate.
3. Decant as much of the supernatant as possible (without losing any solid material from the container) into a squirt bottle. This bottle should be labeled "Formalin". You will use this formalin seawater mix in the squirt bottle through out the handling of its respective sample (see following note).
4. Gently swirl remaining supernatant and solids in its host container and then pour into the sample splitter and split the sample by rotating the splitter wheel back and forth several times to split the sample evenly (Fig. 2). When finished pour the sample material into the splitter containers by tilting the wheel back (Fig 2). Use the trap supernatant in the squirt bottle (conservatively) to loosen solids stuck inside the splitter wheel. You now have two half splits. **NOTE:** Throughout the splitting process, you will need to use the supernatant in the squirt bottle to clean out the splitter and other containers/decanter used in this procedure. Make sure to use supernatant conservatively, but it is necessary to clean thoroughly at each step in the process.

5. Take 1/2 of this first split and decant into the appropriate sized Nalgene bottle (250ml). This is your 1/2 split archive. Label the bottle with the trap sample name and "1/2 Split Archive" and refrigerate.
6. You will now split the remaining 1/2 again. Place the half split into the sample splitter again rotating it back and forth several time and pour into the splitter containers. You now have two 1/4 splits.
7. Take each 1/4 split and decant into a tray or large petri dishes if necessary.
8. Use forceps to pick swimmers and other things (particularly "snotty" things) from the two 1/4 splits that might gum up the filtering process. Be sure to pick each 1/4 split as evenly as possible. Place the pickings in a plastic media dish on a pre-weighed Nuclepore/Milipore filter. Label the media dish(s) the trap cup information and "1/2 split pick" and the sample name, and put it in oven (60°C) to dry.
9. Take one of the 1/4 splits (contents of one tray) and decant into 50ml centrifuge tubes. Run these in the centrifuge for 10 min. at highest setting (Fig. 3). After the sample has been spun down once, decant the liquid fraction from centrifuge tubes into your squirt bottle. Consolidate the contents of all centrifuge tubes into one tube using Nanopure water and shake the sample until it is well mixed with the water. Label the tube with the trap sample name and "1/4 split powder" and centrifuge for another ten minutes. Decant the remaining liquid fraction into a waste container and place the centrifuge tube with unscrewed cap in the oven at 60°C to dry. This fraction will be used for chemical analyses such as carbon and nitrogen concentrations and isotopes, weight percent biogenic silica, phosphate, and carbonate analyses. **NOTE:** You should carry out the remaining splitting and filtering while centrifuging sample.
10. Place the other 1/4 split into the splitter again rotating the wheel back and forth and then pour the contents into the splitter containers. You now have two 1/8 splits. At this point you have two possible protocols to follow. If the sample has very little solid material you will continue with step 11, skipping only step 12 and continue to the end of the procedure. However, if you have a large amount of solid material you will skip step 11 and go to step 12 and continue to the end of the procedure.
11. If your sample has very **little solid material** decant one of the 1/8 splits into a clean Nalgene bottle or beaker and set aside. Place the other 1/8 split portion into the sample splitter and split.
 - a. Place three weighed Nuclepore/Milipore filters on the filter frits of the filter rig. Use a little Nanopure water to keep the filters in place then use the clamps to secure the funnels in place. Place the labeled empty media dishes in front of the appropriate filter. Separately filter both 1/16 splits using the first two filter rig setups.
 - b. Decant the remaining 1/8 split from holding container (bottle or beaker) into sample splitter and split. Take one 1/16 split and decant this into the remaining filter rig. Make sure to label the filter media dishes with the sample name and "1/16 split" and turn on the vacuum line to begin filtering.
 - c. Take the remaining 1/16 split and decant into a small Nalgene bottle and label this with the sample name and "1/16 split" this fraction will be used by A. Leventer or another designated investigator. Place this bottle in the refrigerator
12. If the sample has **moderate to a large amount of solid material** you will need to continue splitting the sample. Choose one of the sample splitter containers holding 1/8 and leave the other container in place. Pour one 1/8 split into the sample splitter and split. After splitting you will now have 1/16 in one of the splitter containers and 3/16 in the other (1/8 + 1/16). Continue splitting one side of the sample the necessary number of times until you feel it is near light enough for ease in filtering.
IMPORTANT: Keep close track of the fractions you are working with. It helps to make a diagram of the fractions that result from each successive split in each container. When you have reached a point that your sample is fairly light on one side, take the remaining side (largest amount) and decant this into a Nalgene bottle. Label this bottle with the sample name, the size of the fraction (i.e. 3/16, 7/32, 15/64, etc.), and "Extra Archive". Place this bottle in the refrigerator.

13. Split the remaining fraction again as in steps 10 and 11 but use the appropriate fractions-- *not 1/8 or 1/16*). Set on split aside in a bottle or beaker (recording/remembering the correct split fraction) and other portion back into the samples splitter and split.
 - a. Place three weighed Nuclepore/Milipore filters on the filter frits of the filter rig. Use a little Nanopure water to keep the filters in place then use the clamps to secure the funnels in place. Place the labeled empty media dishes in front of the appropriate filter. Separately filter both 1/XXX splits using the first two filter rig setups.
 - b. Decant the remaining 1/XXX split from holding container (bottle or beaker) into sample splitter and split. Take one 1/XXX split and decant this into the remaining filter rig. Make sure to label the filter media dishes with the sample name and "1/XXX split" and turn on the vacuum line to begin filtering.
 - c. Take the remaining 1/XXX split and decant into a small Nalgene bottle and label this with the sample name and "1/XXX split" this fraction will be used by A. Leventer or another designated investigator. Place this bottle in the refrigerator
14. As filtering progresses use a little Nanopure to clean off any sample that may accumulate on the side of the filter funnel glass and to wash salts off the filter.
15. Once filtering is completed, remove filter from frit and place in the appropriate labeled media dish. Place in the oven at 60°C to dry.