

Operation of the Barger Lab Gas Chromatograph For ARA (Acetylene Reduction Assay) Experiment

Introduction:

Following an ARA experiment (See ARA protocol), it is necessary to measure the amount of acetylene fixed into ethylene on a gas chromatograph (GC). This method outlines how to run the Barger Lab GC, including set-up, calibration curve, and some troubleshooting.

Materials

Hewlett-Packard (HP) 5890 series II gas chromatograph
Hydrogen tank with more than 500 psi of gas
Zero air tank with more than 500 psi of gas
Ethylene standard (10 ppm) tank
Vacuum pump connected to the GC
30 mL Syringes
60 mL Syringes
3-way stopcocks
22 gauge syringe needles
Exetainer samples from ARA experiment
Lighter

Run-time Instrument Settings:

Oven: 40°C

Detector B: 320°C

Injector: 200°C

Turning on the instrument:

1. Turn on the Hydrogen and Zero air gases (at the tank only, it is not good to mess with the settings on the regulators themselves)
2. Turn on the instrument (on switch is located on the bottom right of the instrument).
Make sure that instrument passes the self test
3. Turn on the vacuum pump (small toggle switch is located on the left near the injection port).
4. Press the **Injector A** button and check that it is turned On. When it is on, values will appear on the GC screen.
5. Press the **Detector B** button and repeat step 4.
6. Press the **Oven** button and repeat step 4.
7. ONLY light the FID when all components are up to the correct temperature!
8. To light the FID, lift up on the top panel of the instrument
9. Press and hold the "FID Ignitor" button and use a lighter to light the FID valve. This is the circular part underneath the hood. Once FID is lit, DON'T put anything on the top of the GC it is very hot and will either melt your stuff or start a fire.

10. To check if the FID valve is on press the **Signal 1** button. The signal value should be around 7-9 on the GC screen (but this value can fluctuate, so don't worry if it not exact). If there is no signal try lighting the FID again.
11. Press the oven temp button and then turn up the oven heat to 150 degrees.
12. Wait ten minutes for the column to bake out.
13. Make sure that the Peak Simple data device is turned on.
14. Turn on the computer and log in.
15. On the computer open the program **Peak 3.77**. There should be two viewing windows open. The top one is the live feed from the GC and the bottom one is for manual integration of the curve.
16. Press the **Oven Temp** button and set the oven to 40 degrees.
17. When the oven is set at 40 degrees, press **Start** to run a blank (room air) through the instrument. You can only run samples when the green light is illuminated on the instrument, indicating that the instrument is ready.
18. The blank should have a completely flat line. If it does not, heat the oven back up to 150 degrees for an additional 15 minutes and re-run another blank.

When you are done running the instrument leave at 150 degrees. If the instrument is not going to be run for a long time, it is best to shut it down so that you don't run out of gases. Running out of gas is a bad thing, so monitor the tanks as closely as possible.

Calibration Curve:

Make sure the valve switch box is in the "load" position to start

1. Open the Ethylene tank at the tank, the regulator should preset. Using the regulator shut-off (not the pressure regulator) purge ethylene from the tank for 10seconds.
2. Using a 30 ml syringe fitted with a stop cock, extract 10 mls of gas from the 10 ppm Ethylene standard. Make sure that you let the Ethylene purge the tube every time you pull a standard. Fill the syringe and shoot it out into the room twice before you are ready to inject a full syringe into the instrument. Take the ethylene standard to the GC and turn the vacuum pump into the hold position by flipping the toggle switch 90 degrees from its previous position.
3. Inject the 30 mls of standard into the GC.
4. Immediately following injection press **start** on the GC.
5. Repeat these steps with a 1 ppm ethylene standard made by filling a 30 ml syringe with the 10 ppm standard, ejecting the ethylene so that there remains only 3 mls in the syringe, filling the rest of the 30 ml syringe with air.
6. With the blank, 1 ppm, and 10 ppm values you can make a calibration curve.

Using the Software:

1. Once the software is open. You should first click **EDIT** button and click on **Manual Integration**. This will allow you to manually integrate your ethylene peaks.
2. Click **Edit** again and then **Channels**. Under channels make sure that channel 1 and channel 2 are open. **Channel 1** should have the **Active** and **Display** boxes check marked. **Channel 2** should have the **Display and Integrate** boxes check marked.

3. Under the **Channels** window you can click on **Details** to change the viewing window. For both channels the best viewing window should be at 10 Hz sample rate, Max 20.000 mV and min - 5.000 mV.
4. After each sample runs you can integrate the ethylene curve by clicking the open folder icon on the top left of the window. Samples are labeled sequentially which makes it easy to find your sample.
5. Open your sample in **Channel 2**.
6. Zoom in on the ethylene peak which is highlighted in red.
7. Check to see if the automatic integration baseline is accurate. Accurate means that it does not over or under-integrate the peak. This takes practice and supervision to adjust.
8. To adjust the baseline curve click the icon on the left with two peaks and a red line with a point at each end. It is the lowest icon with a red line.
9. Using the arrow, you can now draw your own baseline integration line. This line should include all of the peak, which can be difficult. Be consistent in the drawing of all of your lines. Practice with calibration curves and check standards to make sure you are consistent.
10. Each sample will have a series of peaks to be analyzed. The software should already have a red line underneath the ethylene peak.

Running Samples:

1. To run samples your exetainers must be overpressurized with your gas sample, usually it is good to start off with twice as much sample as container volume.
2. To get the gas from these samples you will put a 22 gauge needle on the stopcock of the syringe and insert the needle into a sample.
3. Open the stopcock and fill the syringe about 7 mls full of gas sample.
4. Close the stopcock and remove the needle.
5. Attach needle to injection port. ***Make sure the stopcock for the vacuum is in the shut off position prior to injecting sample***
6. Inject the sample into the GC.
7. Press start on GC panel.
8. Repeat this with 10 samples and run an ethylene standard.

Adjusting Gas Flow:

There are two flows that need to be set, the column flow and the FID flow. The column flow should be around 5-6 and the FID flow should be around 34-36.

1. Turn off FID air and H₂ on the left hand panel on the GC. Use the flowmeter (located near the CN analyzer) attached with the flow fitting. The FID flow fitting fits right into the hole of the FID. Make sure this is seated in there properly. Check flow. This will be the column flow of H₂ which should be around 5-6ml/min.
2. Turn on FID air for FID flow test. Leave H₂ flow off for this test. FID flow should be around 34-36ml/min and can be adjusted directly at the regulator if this is low.