Gas Chromatography (headspace samples) using HP5890 machine

Hazards to be aware of before starting:

- **Hydrogen gas is very flammable**. If you don't set up the GC correctly, it is possible for unburnt H_2 gas to come out of the detector or (worse) accumulate in the oven compartment, posing a risk of fire or explosion. Ensure GC columns are installed properly (this is the job of the boss of the lab), and the detector is lit properly before commencing work.

- Other **GC gases such as nitrogen and helium are asphyxiants** (cause suffocation). Ensure that these gases are all plumbed into the GC correctly, and that there are no leaks.

- **Pressurised gas cylinders (H_2, N_2, air, helium) are hazardous,** especially if mishandled or dropped. There are two major risks here. Firstly, if the top of the cylinder is knocked off by a fall, the cylinder can launch like a rocket and go thru walls etc. Serious injury risk! Secondly, if the cylinder ruptures in a confined space, there is an asphyxiation and/or fire risk, depending on the gas. Don't handle gas cylinders without first having hands-on training from the lab boss !

- **The top of the GC is hot**, especially the injector port and the detector. Don't get burned !

- **Needles used for injecting samples can cause an injury**. Needlestick injuries pose an infection risk in a lab setting, especially if there are microbes or soil etc on the needle.

- Many of the **hydrocarbons or chlorinated hydrocarbons** that we analyse using the GC are either flammable or toxic or both. Learn the risks of these chemicals before starting work.

Turning on the GC

- Ensure the GC computer is turned on (if you turn on the GC without access to the software, the GC will auto-shutdown)

- Turn on gas cylinders using the knobs on the top of the cylinder. You don't need to turn the knob very far (half-a-turn is fine). Don't change the pressure setting – this is the other knob that you will see, and is on the side of the gas regulator.

- Turn on all the four gases at the control board behind the GC – these taps need to be running parallel to the direction of the gas lines. Only turn on gases that are actually connected to a GC machine (follow the gas lines to see where they lead if unsure).

- Turn on the gases using the knobs on the left hand side of the GC machine: Helium, hydrogen, nitrogen, air. Turn on the taps (anticlockwise) approx. 3 turns. You are now committed to igniting the GC detector – don't walk away! Do the next two steps quickly.

- Turn on the GC power on the back of the right hand side of the machine.

- Press in the FID (flame ionisation detector) ignitor button once. As you do this you should see a flame flash inside the detector on the top front right of the GC (gold ring), and you should hear a 'pop' sound. Check for condensation above the FID to confirm the detector flame is lit (use a shiny metal spatula or equivalent to look for water droplets, indicating combustion of $H_2 + ½O_2 \rightarrow H_2O$). You can also check for signal (press 'SIG' button) – this should be around 20-30 at baseline if the FID flame is lit. If its <1, its not on. Sometimes blowing gently across the top of the FID helps to light it (really!). If you don't have ignition, and can't resolve the problem quickly, turn off the hydrogen gas, and then seek help.

- Once the GC display reads ‘passed self test’, double click on the ChemStation software icon on the computer. The display on the GC will flash quickly as information from the software is inputted. Once the display stops flashing the GC will begin to carry out the instructions set by the software. This is referred to as the method and controls the oven temperature, injector pressure etc. for the GC. There are two figures for each of these parameters- the set point and the actual. Once the actual equals the set point the machine will be ready for use and will say so on the software and on the GC display.

- **NB.** If the GC cannot carry out the instructions inputted from the software it will start beeping and eventually auto-shutdown (‘EPPA safety shutdown’). If the machine starts beeping check the Injector Pressure A (‘Shift’ (yellow button) and injector A temperature) and the Signal (both on the right-hand front face of the GC). For most programs the Injector A pressure should be 20. The Signal should be ~ 20. If these two parameters differ from these there are some things you can do to try and fix them:

  - Turn the Total Flow (Helium) on a few more turns.

  - Replace the septum (red round disc) which you inject your sample into (left front side of the GC top with a green metal cap on top). Regardless, this should be done after numerous sample injections as the septa will get more needles holes in it and hence not keep pressure. If you have been using the GC then the Injector A will be very hot so be careful. You can replace the septa while the GC is on provided you are quick.

  - Turn off and on the GC (including all gas inputs and gas bottles).

- **The GC is ready for use once the GC and ChemStation software display ‘Ready’**
  - Once all of the ‘actuals’ for oven temperature, injector A pressure and temperature are at the set point and the ‘Signal’ is around 20 the GC is ready for use. The green ‘Ready’ on both the software and GC also indicate this.

Running a method

- A method is what the software tells the GC to do. Two common methods that we use in the lab are “ETHENE.MTH” and ‘CHLORO2.MTH’, respectively. These methods have been designed so that compounds of interest can be separated from one another in a minimum amount of time. The main variables changed by different methods are the oven temperature (max 240°C) and the run time (usually between 5-20 min)

- The method used last time the GC was used will automatically be run when you turn on the GC. To change the method select ‘Load’ on the Method tab. If you edit a method (using ‘Edit entire method’ in the Method tab) you must press ‘Save’ afterwards. Selecting ‘OK’ after you have made all of changes in the ‘Edit entire method’ dialogue box will not save the method.

- Different compounds bind with different affinities to the column and so are eluted off the column at different temperatures. The retention time (RT) is the time at which a compound is eluted off the column. Once the compound is eluted off the column it passes through the hydrogen/air flame which breaks down organic compounds and produces ions. The ions are collected on an electrode which produces an electrical signal.

- The ChemStation software presents the run as a series of peaks which indicate the retention time (and so the identity of the compound) and the area of the peak. **NB.** You are interested in the peak area, not the peak height.

Running a sample

- Before starting, figure out if you need to use sterile needles or not. For live growing cultures, you should be using sterile needles, ie. a new needle for each sample. You will need two small glass beakers, one for sterile needles (foil-covered, autoclaved), and one for used needles. These needle beakers will rotate duties between clean and used. We use removable needles with point-style 5 (side-port). They are expensive, so take care of them. Don't flame them. For resting cell suspensions or cell extracts or standard curves it's OK to use a non-sterile needle, ie. the same needle for multiple samples.

- Before injecting each sample, make sure you change the Sample Information in the ChemStation software – go to the tab Run Control -> Sample Info. Use concise sample names eg. 12janE11 might be the first sample for ethene on January 12th. If you don't change the Sample Info for each sample, the previous file will get overwritten.

- Before injecting a sample, make sure your samples have equilibrated. It takes time for a gas-liquid equilibrium to establish for volatiles. Depending on experimental setup, you need to balance the need to equilibrate with the need to sample as early as possible. The minimum would be to pre-incubate the bottle / liquid /cells etc at the correct temperature, add substrate, shake/ hard by hand for 10 seconds, then re-incubate for 5 min.

- Take your sample bottle from the shaker and withdraw a 250μl sample using a 250 μl glass, gas-tight syringe with a side port needle. Sample only the headspace, do not sample from the liquid phase. Remove the syringe and lock the sample in (by clicking in the red button). Replace the sample bottle in the shaker.

- Insert the needle fully into Injector A (green metal top). Push in the green button on the syringe and inject the sample in a single smooth rapid motion, then immediately press the 'Start' button on the GC.

- Keep the depressed syringe in the injector port until you see the ChemStation software displaying 'Run in progress'. This indicates that the sample has entered the column.

- Withdraw the needle – when doing this, hold it by the hub of the needle itself, not the body of the syringe, otherwise you risk the needle coming off the syringe – if this happens, your sample can blow back out the needle. Also beware of touching the hot injector port! (200°C)

- Once the run is finished, the software will output the results. If the results screen doesn't pop up automatically, you can navigate to it in the "Results" tab. Note down relevant retention time(s) and peak area(s) for the compounds of interest in your lab book.

- Troubleshooting – no peaks or wrong/variable peak areas:
    - the needle may be blocked, usually with red rubber material from inside the septum; you can use a fine wire to clean it - these are found in the wooden drawer of GC stuff.
    - gas may be escaping because the white tab on the end of the plunger is not tight. Test this by sucking in some air, pushing in the red locking button and depressing the plunger. If the plunger moves back at least some of the way to the starting point, you have a good seal. If not, unscrew the inner plunger and tap the white Teflon tab several times (quite hard) on the bench surface and retest. When tapping the plunger, support the shaft with several fingers so it doesn't bend. Its good practice to do this plunger-tapping before starting each GC session.
    - gas may be escaping due to foreign matter (grit, dust, soil etc) in the syringe body. you can clean by squirting water into syringe, but ensure it is fully dry before re-using.

Standard curves for gas chromatography

- Standard curves are needed to relate peak area to amount of substrate injected. The lab has existing standard curves which may be applicable to your project, but usually due to differences in experimental setup and changes in GC response over time, it is best to make your own standard curve.

- To create a standard curve, make up 5 bottles, each containing different amounts of volatile substrate (or mixture of substrates, provided they can be separated by the GC). Make sure that the standards over the entire range that you will be working over. We usually make standards in 120 ml serum bottles containing 30 ml of RO water. Allow the standards to equilibrate for at least 30 min at the incubation temperature your other experiments will be done at before sampling them (temperature affects the partitioning of volatiles between gas and liquid, so a standard curve made at 20°C is not applicable to cultures incubated at 30°C).

- For each standard bottle, make at least 3 injections, and run on the GC as above to collect the data. You should be able to get less than 5% peak area difference between replicates. If your errors are much more than 5% then you are doing something wrong, or the equipment is faulty (most commonly the syringe plunger is not gas-tight).

- Plot all your data and create a graph with peak area on the y axis and amount of substrate in micromoles on the x axis. The resulting trendline should be linear – you should be able to get r^2 values of at least 0.99 if you are careful. You can then use this equation to calculate amounts of substrate in unknown samples, with the following rules:
    - The unknowns must be at the same temperature
    - The unknowns must be in the same size bottle with same volume of liquid*
    - * but note that its OK to use the same std curve if the **ratio** of gas to liquid is the same in your test sample e.g. a standard curve made with 30 ml liquid in 120 ml bottles can also be used for 4 ml samples in 16 ml bottles.
    - The unknowns must be run within approx. 3-6 months of the standard curve (detector response and other factors change over time, and a new std curve is required)

- Don't throw out your standards! If you look after them, they should stay good for many months, and are valuable positive controls if you have concerns about your syringe function or any other aspects of your GC performance. Best practice would be to keep your standard curve bottles wrapped in foil, at 4°C, and inverted inside a beaker or something (so that liquid covers the lid). Make sure to let them equilibrate at your sample temperature before use however (at least 1 hr if they were at 4°C).

Shutting down the GC

- The GC is switched off more-or-less in the reverse order to turning it on. Since high temperatures can damage columns if they are run without carrier gas (helium), it is best practice to first lower the oven temperature manually to 50°C (hit Oven Temp button on GC, then set to 50, then press Enter). Once the oven has cooled, switch off the GC at the power button at the back right hand side. Then switch off all the gases on the GC itself, then on the regulator board at the back, then at the cylinders. It's OK to leave the computer running.

GC maintenance

- In times of heavy use, the GC column should be cleaned approx. once per week (raise temperature to 240°C for 1 hour to burn off gunk). Also the septum in the injector port needs to be changed at about the same frequency.