

GC/MS Discovery Laboratory
“How is molecular structure related to odor?”
Physical Chemistry C131

Purpose

Isolate and identify compounds that result in odor. Once the chemical components have been identified, compare their characteristics with other known chemicals with a distinct odor. Identify one or more important characteristics of the molecular structures of the “smelly” compounds identified.

Background / Introduction

An important aspect of physical chemistry is the exploration of the fundamental relationships between the structures of molecular compounds and their physical properties. In this lab you'll examine how odor and functionality are related by identifying the chemical components of a smelly compound. Obviously one characteristic that all smelly compounds must share is the ability to enter the gas phase and reach your nose. A class of compounds that can do this are known as volatile organic compounds (VOCs). What is it about some of these compounds that gives them a high vapor pressure and a distinctive smell?

Readings on odor:

E. L. Saul, *J. Chem. Ed.*, **23**, 296-297, 1949.

A. M. Rouhi, *C&E News*, **80**, 24-29, Jan. 7, 2002.

Readings on mass spectrometry:

M. M. Campbell, O. Runquist, *J. Chem. Ed.*, **49**, 104-108, 1972.

D. A. Skoog et al., *Principles of Instrumental Analysis*, Fifth Edition; Saunders College Publishing: Philadelphia; 1998, pp 258-260, 498-505, and 524-528. Information on quadrupole mass spectrometers, electron impact ionization, and spectra interpretation.

Materials and Instruments Needed

SPME fibers, 7 μm bonded polydimethylsiloxane (pre-conditioned) and/or DVB/CAR/PDMS

Gas tight syringe

Vials, 20 mL with silicone septa and cap closure

GC/MS

Cappillary Column, 30m \times 0.25mm i.d. \times 0.25 μm RT_x-5 (95% dimethyl- 5% diphenyl polysiloxane), or DB-5MS from J&W

Merlin microseal

Procedure

You will have two laboratory periods to obtain data to answer the above question and the third period will be used to analyze and interpret your data as well as to begin to prepare your presentation. As a team you will choose natural or synthetic products that have a readily identifiable smell. Your goal is to isolate and identify the chemical components

that result in the odor. Before we begin the lab hypothesize as a group as to what you might expect to identify in the chemical structures of smelly compounds.

In the laboratory you will be introduced to the technique of solid phase microextraction (SPME) which allows the sampling of either the vapor above a liquid or solid (headspace) or an aqueous sample itself to be sampled. You will also be introduced to the gas chromatograph mass spectrometer (GC/MS) and its use. Once a sample has been absorbed onto a SPME fiber, the individual components can be separated using GC and the mass of each peak can be identified using MS. The National Institute of Standards and Technology (NIST) databases will be used to help identify the components in your sample. You will need to develop a procedure of how to spend your time after the introduction on the use of the instrument.

Remember that doing a literature search and running standards are commonplace research activities and are expected during this lab. Your goal is to identify the three largest peaks in each mass spectrum of each compound you analyze. This may necessitate determining the chemical structures and the most likely fragmentation patterns of the chemicals in the compounds analyzed.

Lab Presentation

A collaborative presentation involving each team member is expected for this laboratory. The presentations will take place on December 3rd during the laboratory period. Each group will have 30 minutes to present their findings. The presentation will be worth as much as a formal lab report and should include all the same information such as an introduction, experimental section, etc. Access to a computer will be provided and it is expected that some of your presentation will be given in PowerPoint.

In general your presentation will be graded on the following criteria:

- a. Preparation. Did the presenters prepare adequately ahead of time? This means doing background reading on the experimental technique used and the system under study.
- b. Delivery. Diction, grammar, volume, and time limits are important. Practicing your presentation as a group beforehand will help.
- c. Clarity. Was the material explained so that it is understandable to an undergraduate physical chemistry class?
- d. Response to questions. This is a measure of the presenters' understanding of the topic and ability to use that understanding "on the spot". This "thinking on your feet" is necessary for job interviews and graduate school orals.
- e. Handouts (enough abstracts for everyone in the class plus a few visitors) and visual aids (PowerPoint). Are they understandable and relevant? Whenever using PowerPoint bring a backup copy of your slides in case of technical difficulties. In this case you will turn in your backup copy (may be paper) to the instructor after the presentation.
- f. Correct use of chemical terminology in reference to techniques used.

g. Interest and enthusiasm. If you are bored with your presentation the audience will be even more bored!

In particular your presentation for this lab should include a theoretical background of GC/MS, operating principles of SPME, information on obtaining samples, the chromatograms and mass spectra obtained, and interpretation of the mass spectra and its major peaks. Also, include an examination of the structures of the compounds identified and any similarities among them. Are there any commonalities among the smelly compounds? Does this match your predictions?

Familiarization with GC/MS

GC/MS is a very powerful technique used widely in research laboratories. In this lab you will have a chance to use this state-of-the-art instrument. To ensure that each student has some understanding of how to operate the GC/MS, we will do a few short exercises. Please note all of your answers to the following questions in your lab book. You will need to copy and turn this in before you begin on the exploratory part of the lab.

First, there are a few instrument safety precautions to appreciate when using this instrument. Do not use the instrument unsupervised. The GC/MS is configured so that Injector B is connected to an Elite 5MS column, which in turn is directly connected to the mass spectrometer. **It is important to ensure that the column has a He flow through it whenever the GC oven is on.** A flow of 1 mL/min is typical. There is an ECD (Electron Capture Detector) that would be used with a second column connected to Injector A. Currently, there is no column attached to Injector A. **Do not attempt to inject a sample into Injector A.** Ensure that the ECD is off (see step 7 below). When the instrument is not in use, it should be left with the pumps on and the filament off. If the instrument is idle for longer than 1 week, decrease the inlet line temperature and the source temperature from 180 to 100 °C.

Your sample will be manually injected into column B. Various components in the sample will be retained on the column for differing amounts of time, just as in the Henry's law lab. A peak in the TIC (total ion count) indicates that a component has eluted from the column and been detected by the mass spectrometer at a particular delay time. A mass spectrum of each TIC peak is then available (right mouse click on the TIC peak to show the mass spectrum of that peak). To begin the identification process of a peak you will compare your mass spectrum to the mass spectra available in the NIST database using the F1 key.

Answer all questions in your labbook.

1. Sign the logbook with name and date.
2. The first thing to check is the pressure. What is the pressure on the Pirani gauge? What is the pressure on the Penning gauge? What is the maximum pressure at which you can proceed with your experiments (Tutorial p.54)?
3. Every time that the instrument is used it is necessary to check the ratios at m/z 4, 18, 28, 32. What does z stand for? What are these masses? Which mass do you expect to be the highest? What should the optimum ratio be? Examine pages 82-83 in the Tutorial manual. If there is an air leak, what masses do you expect to be the highest? Turn on the filament and high voltages (press for operate) and write down these ratios.

4. From the tune page ensure that you are working from the D drive in the P. Chem. Project. Do a save as before beginning with today's date. We will now tune the instrument. This does not need to be done every time. Follow the instructions on pages 105-109 in the Tutorial. Make sure that the reference gas is not being pumped out (i.e. Tune page → Gas → "pump out reference gas" should not be checked). Then turn on the reference gas. Be sure to do a maintenance tune and not a full tune. The mass spectrometer is tuned by letting in a small amount of perfluorotributylamine (C₁₂F₂₇N) gas as a reference. Set the peak editor for m/z = 69, 131, 219, 502 (p.72 Hardware manual). What are the fragments at these four values of m/z? Print out a copy of your tune results. Check that the masses are calibrated, i.e. the middle of the peak should be centered. Sometimes when doing an autotune it is necessary to press stop and then start again if the ramp does not start right away.
5. Now we are going to vary some of the parameters to see how they impact the mass spectrum. Go to the peak editor on the Tune page. Decrease the LM resolution (left side of Tune page). What happens to the peaks? Decrease the multiplier. What happens to the peaks? Pick a few other parameters and observe what happens when they are changed. Examine the peaks closely. It may be necessary to increase the gain in the peak editor. Do you see a second smaller peak? What is this smaller peak due to? Do a second tune (remember to do a 'save as' first) and change the peak width at half height from 0.6 to 0.5. What happens to the valley between the peaks? Why would an increase in resolution (spacing between peaks) lead to a decrease in sensitivity (decrease in signal level)? Return to your first tune. You will take spectra using this tune. Shut the reference gas off. Pump out the reference gas and then shut off the pump.
6. Smash up a cherry lifesaver. Places small pieces of it in a vial and allow to equilibrate while you are setting up the GC and MS methods.
7. The GC method determines the injector temperature, carrier flow, and oven temperature (TurboMass page → GC → method editor, load a new method, use instrumentation → instrument control). It is recommended to set injector B at 250 °C, the carrier flow at 1 ml/min, and the oven temperature at 40 °C for 7 minutes. Ramp the oven temperature to 140 °C by 20 °/min. This should give you a GC method that is 12 minutes long. Ensure that the ECD is off and no carrier gas flows are going to column A. Note that you will be doing a manual injection.
8. Develop a MS method (Tutorial p 127). You will be injecting a 1 mL headspace sample initially. If necessary, you may use SPME for your own samples to achieve greater sensitivity and injection reproducibility. It is important that the MS filament not be exposed to the air that comes directly through the column. Under the settings above air comes through at about 0.73 minutes. Set a solvent delay of 2 minutes to ensure that the filament is off during this time. Turn the MS on from 2-12 minutes and choose a mass range. Initially a mass range of 40-300

- may be useful. You could scan from 10-300, however, that will dramatically increase your background. Why does this increase the background?
9. Once you are satisfied with your methods, create a sample list (Tutorial p143-146). Name your first sample x_01. Press start and then run. When the GC reads ready (oven should be at 40 °C), inject 1 mL of your headspace and press run on the GC. Update your chromatogram in real time (Chromatograph→display→ real time update). At what time do you see a peak? How many peaks total are there over 12 minutes?
 10. Now we will examine some of the peaks more closely. This information will be very useful to you when you try to test your hypothesis on odor and functional groups. Select the earliest peak and examine its mass spectrum (right click on peak). Why are there so many peaks in the spectrum? Use the F1 key to compare it to the NIST library. What compound gives you the most hits? Draw its structure in your labbook. Do you think this makes sense? Is the assignment to this structure definitive, or could your compound have the same mass but different structure (isomer)?
 11. A very useful feature of the MS program is that you can pick certain masses to be displayed. (Chromatograph window→display→mass, type in mass of interest) Pick masses 91, 77, and 105. Why would we look for these masses? What functional groups are they? Are there any peaks on the TIC that contain these masses? Do all the peaks? Can you think of other functional groups that you might look for?
 12. Shutdown procedure: when finished running samples, be sure to turn off the filament before leaving.