GC Pre- and Mini-lab Activities: An Overview

The GC pre- and mini-lab activities to be described below accomplish three important educational goals. First, it's common practice in universities and colleges with large numbers of students per lab session that, prior to the first experiment using a GC instrument, students are given a reading assignment or hear a lecture that describes GC instrumentation and the factors influencing retention time. Students then more or less take it on faith rather than experimental evidence that a substance's molecular weight (MW), boiling point (BP), and the GC oven temperature (T_{oven}) influence retention time (R_t) and hence peak resolution in a gas-liquid chromatographic separation.

The Department of Chemistry at Arizona Western College (AWC) takes a different approach than that described above. Rather than reading or hearing about how a GC works and taking that information on faith as mentioned above, students first speculate about what factors go into designing such an instrument. Second, students gain valuable hands on experience using the GC weekly, not just once or twice a semester. Third, each injection of a liquid requires in turn an interpretation or analysis of the chromatogram. In short, do the data support or contradict one's speculation? Data analysis is a key component in the education of future scientists and engineers.

Based on anecdotal evidence accumulated by the PI teaching college chemistry for eleven years, students oftentimes have a "black box" mentality when it comes to using and understanding instrumentation. Many students think (erroneously) that instruments work on magic rather than on understood scientific and chemical principles. To show students that instruments are not magic boxes, the PI designed some GC pre-lab activities that challenge students to speculate about what factors influence how a gas chromatograph separates compounds in a liquid mixture. Specifically, the GC pre-labs and the accompanying mini-lab that follow a pre-lab focus on what physical factors may or may not influence a compound's retention time (R_t), *i.e.* the time it takes for a compound to travel from the site of injection into the GC, through a capillary column of constant diameter (typically 0.25mm inner diameter) and length (typically 30m), and then exit from the column into a detector (FID- flame ionization detector or TCD- thermal conductivity detector). Factors emphasized in the GC pre- and mini-labs are molecular weight (MW), boiling point (BP), GC oven temperature (T_{oven}), injector temperature (T_{inj}), and, for the more advanced sophomore Organic Chemistry students, the thickness of a capillary column's condensed stationary phase (d_f = density of film), and column polarity (non-polar vs. polar), time permitting for the last factor.

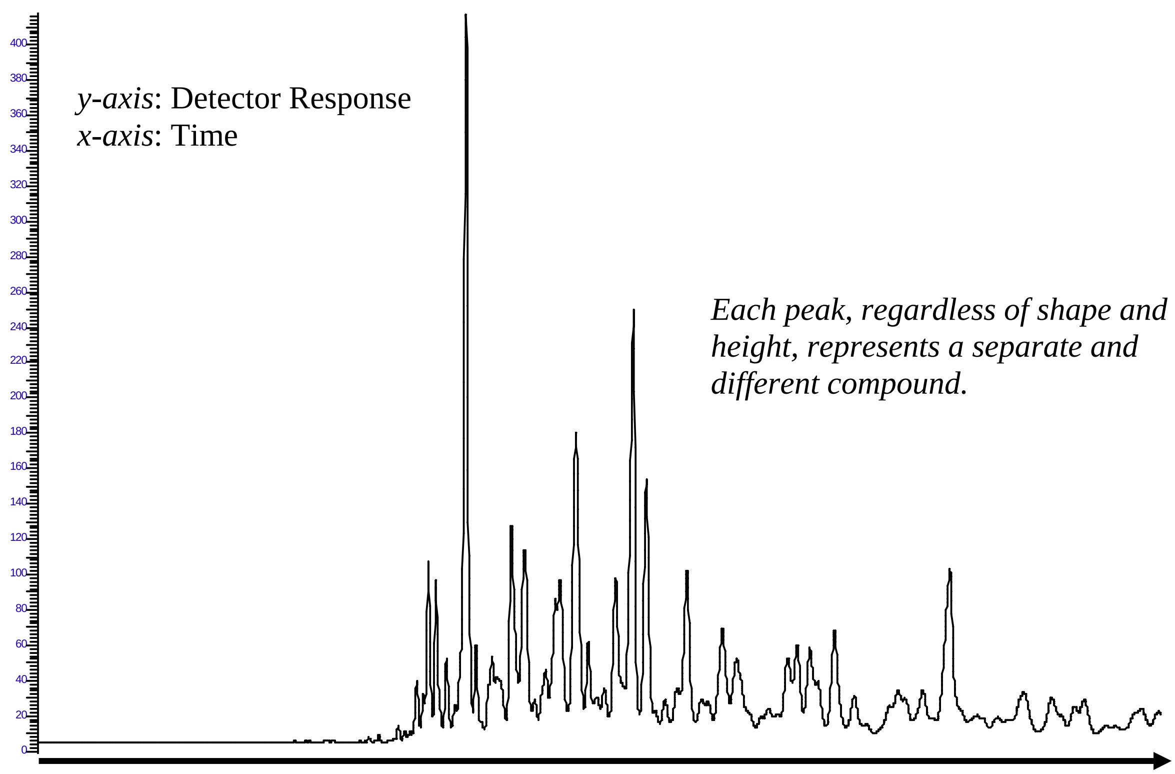
Typically, each GC pre-lab activity lasts no longer than seven to ten minutes and consists of typically two to three conceptual questions, some of which require a short written response. Others are multiple guess or require students to draw a hypothetical chromatogram based on information found in a question. After completion of a GC pre-lab, students then go immediately into the lab and carry out the mini-lab related to the GC pre-lab.

Each mini-lab takes no more than five minutes per student to complete since the PI chose liquids with comparatively low BPs and a T_{oven} that results in elution of compounds in less than five minutes. This pre-lab followed by mini-lab organization allows students to compare their pre-lab results (answers) arrived at cerebrally through speculation to experimentally obtained results in the mini-lab. In short, students first think about what will happen and then actually do a simple “experiment” to find out the answer. For example, the first GC pre-lab asks students to speculate about the potential influence a compound’s MW has on retention time, R_t. Is the relationship direct, indirect, or non-existent? Once a student speculates about it, he/she then goes into the lab and injects (separately) into the GC a minimum of two liquids, say 2-propanol and 2-butanol, with different MWs. Students then compare the retention times and from the chromatogram determine if their pre-lab “answer” is confirmed by the mini-lab experiment, *i.e.* does MW affect R_t and if so is the relationship direct or indirect? In the second GC pre-lab students speculate about the relationship, if any, between BP and R_t. As before after completing the pre-lab each student then injects two liquid isomers 1-propanol and 2-propanol (same MWs) but different BPs. As before students then compare the mini-lab’s experimental results with their speculative “answers” arrived at in the related pre-lab.

GC prelabs 4-6 are geared more towards second semester Organic Chemistry students.

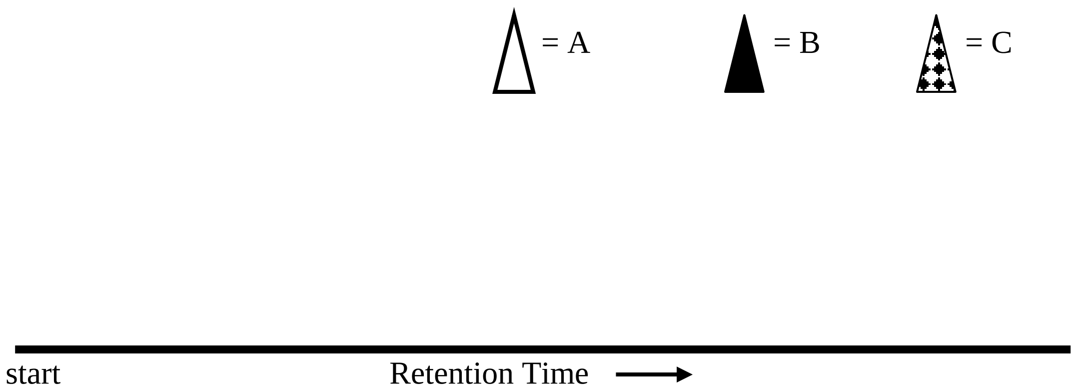
GC-1 Prelab

1) Below is the chromatogram of paint thinner shown in pre-lab. In a chromatogram each peak represents a different compound. What physical factors may contribute to the separation of the myriad number of compounds in paint thinner? List at least three physical factors below.



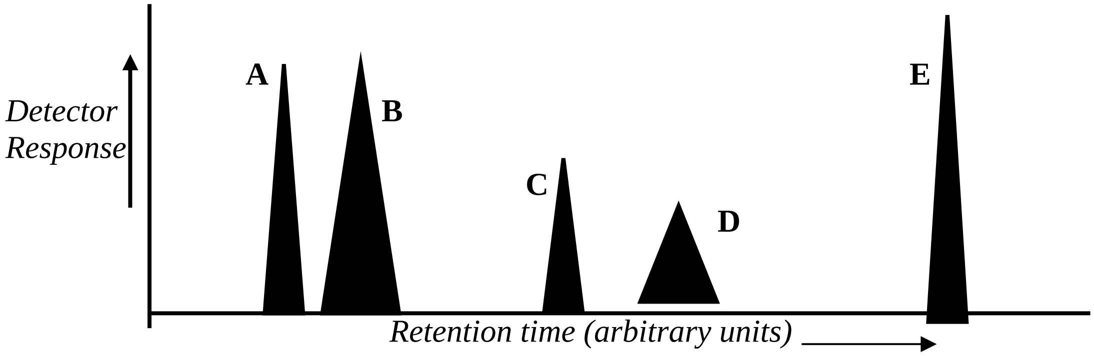
Go to Question 2) but do not return to Question 1)

2) Gas-liquid chromatography, or GC for short, physically separates liquids in a mixture according to a number of factors, one of which is molecular weight, or MW. Suppose a mixture of three liquid hydrocarbons (compounds made up of carbon and hydrogen only)- **A**, **B**, and **C**- was injected into a gas chromatograph (GC) outfitted with a capillary column uniformly heated in the oven to 75^{O}C. The relative molecular weights (MW) of the three hydrocarbons are $MW_C > MW_A > MW_B$. *Retention time* (x-axis) is the difference in time between when a substance was injected (start) and when it exits the GC into the detector. Based on this information arrange the three compounds in the order that they elute or exit from the column.



Go to Question 3) but do not return to Question 2) or 1)

3) Below is a hypothetical GC chromatogram of a mixture that contains five different liquids (**A-E**). *Retention time* is the difference in time between when a substance was injected and when it exits the GC into the detector. Answer the questions below.



1) Which peak represents the compound that travels through the GC instrument in the least amount of time?

____

2) Which peak (or compound) has the shortest retention time? ___________

GC-2 Prelab

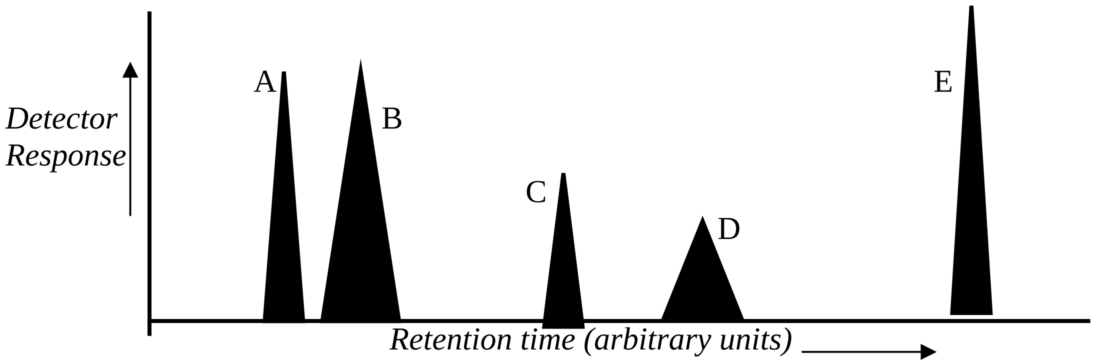
1) A GC analysis can completely separate 1-propanol from 2-propanol, *i.e.* different retention times, R_t, even though the two alcohols have the same molecular weight since each has the same molecular formula C_3H_8O.

```
     H   H  H                    H   H  H

H--C--C--C--O-H             H--C--C--C--H

     H   H  H                    H   O  H
  1-propanol
                              2-propanol
```

Consequently, the difference in retention time (R_t) between the two alcohols cannot be explained on account of a difference in molecular weights since both have identical molecular weights. So what then explains why they have different retention times? *Offer a brief but thorough explanation.*

Go to the Question 2) but do not return to Question 1)

2) As you learned last week gas-liquid chromatography, or GC for short, physically separates liquids in a mixture according to a number of factors, one of which is molecular weight. Suppose you carry out a GC analysis of a bottle that is not labeled. Using a small volume syringe you withdraw some liquid sample from the bottle and inject it into the GC (total volume = 0.1μL). Below is a hypothetical GC chromatogram of the analysis of the unlabeled bottle. Answer the four questions below.



- Which peak represents the compound that travels through the GC column in the least amount of time? ____________

- Which peak (compound) represents the compound with the highest molecular weight?

  ____

- Arrange the five peaks with respect to their relative molecular weights, from heaviest to lightest.

  ______ > ______ > ______ > ______ > _______

- Which peak represents the compound with the shortest retention time, R_t?

  ____

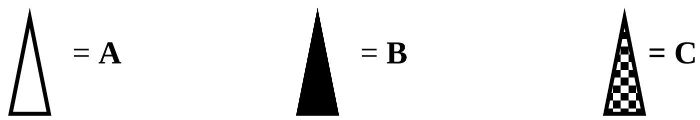
Go to the Question 3) but do not return to Question 2)

3) GC also physically separates liquids based on their differences, among other factors, in boiling point (BP) temperature. Suppose then there are three liquids- called **A**, **B**, and **C**- with different boiling points as shown below.

BP: A > C > B

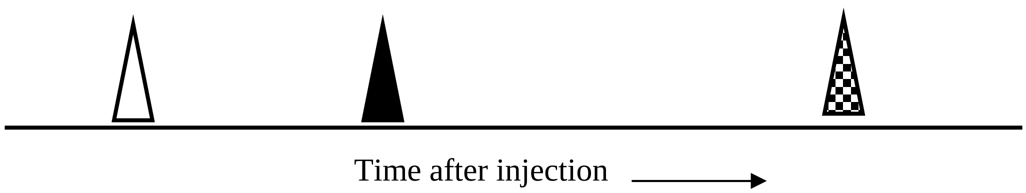
The three component liquid mixture is injected into a GC instrument whose oven temperature is held constant, does not change over time (termed "isothermal" where the prefix <u>iso</u> means *same* and the root word <u>thermal</u> means *heat* or *temperature*). Which chromatogram below (choices **1, 2**, **3**, or **4**) best represents the order in which the three liquids exit from the GC instrument?

Answer: ___________

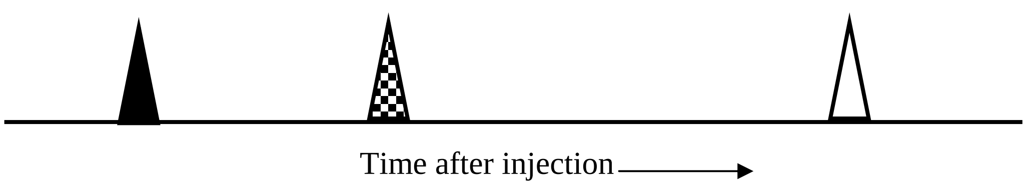


1.

2.



3.

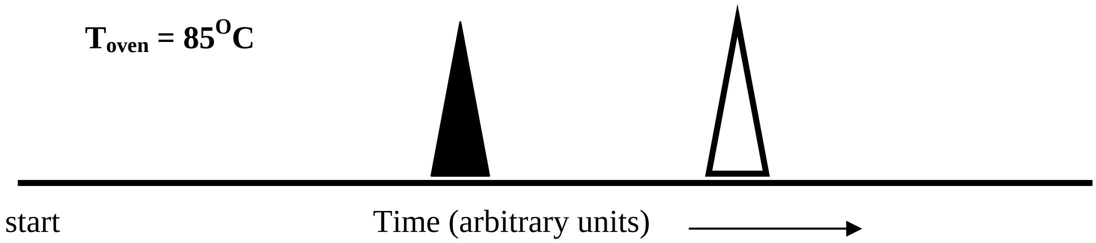


4.

GC-3 Prelab

1) Thus far you have learned molecular weight and boiling point influence a liquid compound's retention time in a gas chromatographic (GC) analysis. In a test tube is a colorless liquid made up of equal moles of compound **X** and compound **Y**. A 0.2μL sample of the two component liquid mixture was withdrawn from the test tube and then injected into a GC whose oven temperature, T_{oven}, is $85^{O}C$. The resulting hypothetical chromatogram is shown below.

$T_{oven} = 85^{O}C$



On the empty x-axis below draw the chromatogram if the same liquid solution was carried out a $T_{oven} = 125^{O}C$.

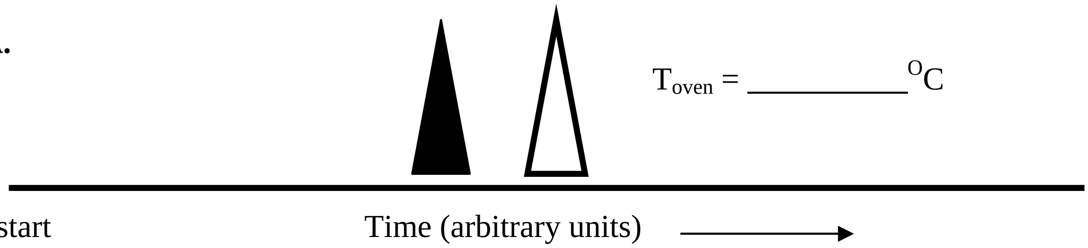
$T_{oven} = 125^{O}C$

start Time (arbitrary units) →

Go to Question 2) but do not return to Question 1)

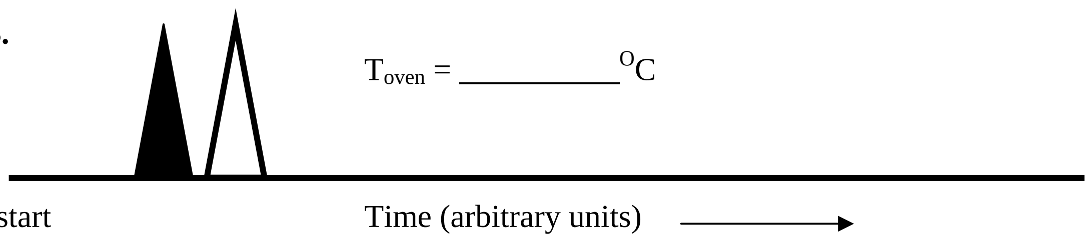
2) Three separate 0.2μL injections of this two component liquid mixture were done at three separate GC oven temperatures- 125^{O}C, 100^{O}C, and 75^{O}C. The resulting three chromatograms are shown below. Match each chromatogram with one of the three oven temperatures at which the chromatogram was produced.

A.



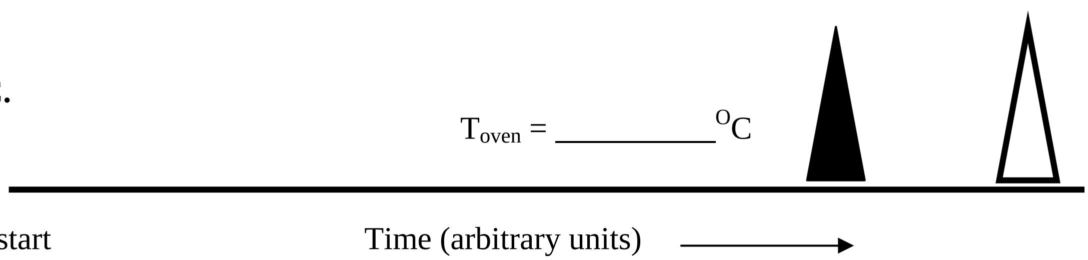
T_{oven} = ___________ ^{O}C

B.



T_{oven} = ___________ ^{O}C

C.



T_{oven} = ___________ ^{O}C

GC-4 Prelab

1) Previously (either last week or last spring semester '06) you learned that the temperature of the GC oven influences the retention time, R_t, of all compounds traveling through the GC column, which is located in the oven. Some compounds though are more affected than others; that is, the increase or decrease in R_t with a corresponding change in oven temperature (T_{oven}) is larger or smaller for some compounds than for others in the sample. This being said, answer the **TRUE/FALSE** question below.

TRUE *or* **FALSE**

The retention time of peaks (compounds) in gas chromatography is influenced by the temperature of the GC oven. The relationship between R_t and T_{oven} is direct, meaning that as T_{oven} increases R_t increases correspondingly.

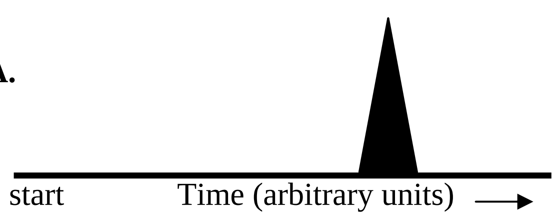
Answer: _____________

2) As you know already in gas chromatography a liquid sample is injected into a compartment (or port) called...you guessed it....an injector. If you touched the injector's metal ring either accidentally or intentionally, you'd likely feel that it's a bit warm, perhaps warm enough to burn the skin. Does the injector's temperature, T_{inj}, influence retention time, R_t? Maybe it does. Maybe it doesn't. Let's speculate.

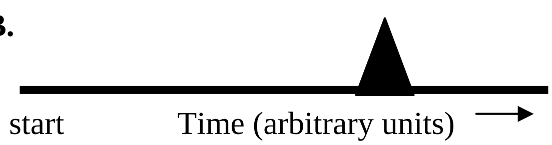
Two separate 0.3µL injections of a colorless liquid compound **X** (b.p. = 135-137^{O}C) were done at the same oven temperature, T_{oven}, but at different injector temperatures- T_{inj} = 100^{O}C and 170^{O}C. Three hypothetical chromatograms are shown below but only two are realistic. Which hypothetical chromatogram (**A**., **B**., or **C**.) represents an analysis done at T_{inj} = 100^{O}C? Which hypothetical chromatogram (**A**., **B**., or **C**.) represents an analysis done at T_{inj} = 170^{O}C?

	Chromatogram letter
T_{inj} = 100^{O}C	__________
T_{inj} = 170^{O}C	__________

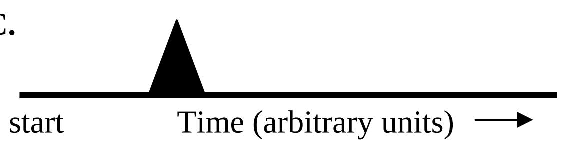
A.



B.



C.



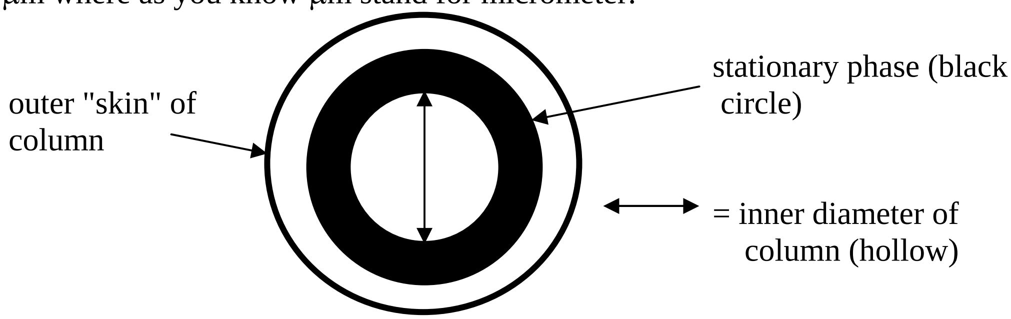
!!Go to next question 3) on back!!

3) As stated previously only two of the three hypothetical chromatograms are associated with the two different injector temperatures. Offer an explanation then for why you did not choose the remaining hypothetical chromatogram.

Explanation:

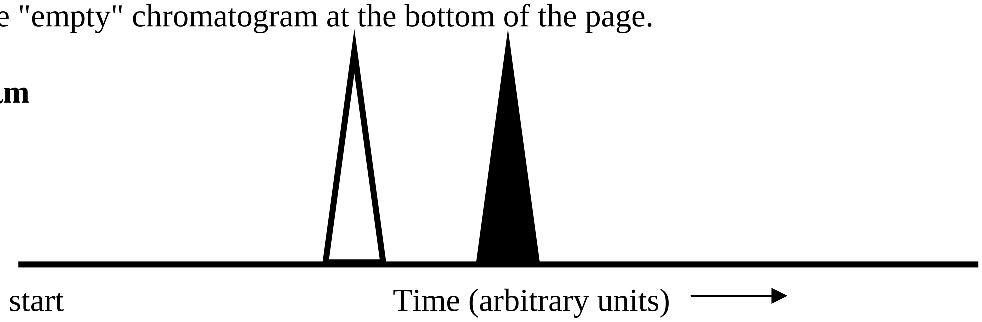
GC-5 Prelab

Below is a pictorial representation (cross-section) of a capillary column (not drawn to scale). The view is one such that you are looking straight down the column. The column's are manufactured so that the thickness of the stationary phase (black circle) can be increased or decreased. Throughout our GC studies we have used the same capillary column (30m long and 0.250mm inner diameter) with a stationary phase thickness equal to 0.250μm where as you know μm stand for micrometer.

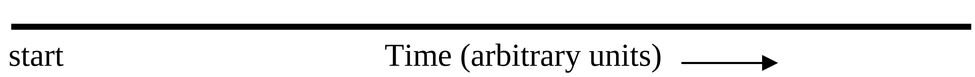


Suppose the chromatogram immediately below is for an isothermal (constant oven temperature) GC analysis of a two component hydrocarbon mixture using a capillary column whose stationary phase thickness equals 0.250μm. How would the chromatogram of the two component mixture look if the capillary column's stationary phase thickness was twice as thick, *i.e.* 0.500μm? The column's length and inner diameter are the same. Use the "empty" chromatogram at the bottom of the page.

0.250μm



0.500μm



GC-6 Prelab

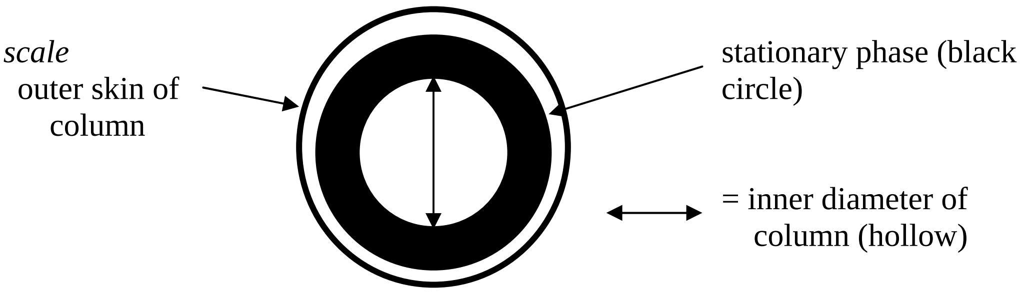
A sample for gas chromatography contains three compounds: 1-hexanol (bp = 157^{O}C), heptane (bp = 98.5^{O}C), and pentylmethyl ether (bp = ~83^{O}C). The structure for pentylmethyl ether is shown to the right.

a) Based on boiling points which compound elutes:

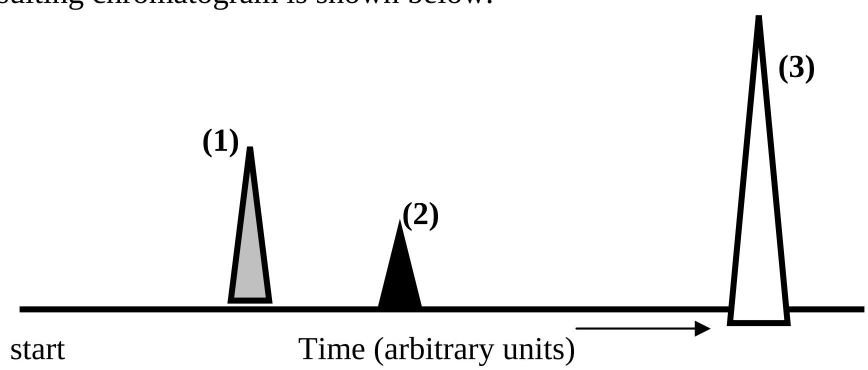
first ________________________________ **last** ____________________________

b) The sample mixture is passed through a gas chromatograph capillary column whose condensed (stationary) phase is chemically composed of a substance that attracts polar compounds. A cross sectional or head-on view of a capillary column is shown below.

**not to scale*



The resulting chromatogram is shown below.



Based on each compound's polarity or attraction to the column's condensed phase match the peak number with one of the three compounds.

Peak Number	**Compound**
(1)	____________________________
(2)	____________________________
(3)	____________________________