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Gas chromatography of a Hydrocarbon Mixture at Two Different Temperatures

Introduction. Gas chromatography (GC) is the method of choice for separating mixtures of volatile compounds, unless they are thermally labile. In that case, liquid chromatography (LC) is necessary.

GC uses a capillary column, which provides especially high efficiency. To a first approximation, compounds in GC separate according to their boiling point as they elute, so the choice of column type is not as critical as it is with LC, but flow rate and temperature are important factors in a GC separation. Gas flow rate is normally optimized by making a van Deemter plot. Column temperature is the factor that we will explore in this experiment.

The effect of temperature can be seen by considering the thermodynamics of the partitioning process that underlies the separation. Volatile compounds are approximately in equilibrium with the mobile and stationary phases as they elute through the chromatographic column, and the ratio of concentrations in the two phases is described by the equilibrium constant, K, termed the partition coefficient,

$$K = \frac{C_s}{C_m} \qquad \text{(eq 1)}$$

where C_s and C_m are the equilibrium concentrations of each analyte in the stationary and mobile phases, respectively. The retention time, t_r, is directly related to the equilibrium constant K through a simple relation.

$$K \cdot \frac{V_s}{V_m} = \frac{t_r - t_0}{t_0} \qquad \text{(eq 2)}$$

where V_s and V_m are the volumes of the stationary and mobile phases, respectively, and t_0 is the time that it would take an unretained compound to elute. Eq. 2 shows the linear relation between K and t_r. One can imagine that K would increase with boiling point, since a higher boiling point will generally shift the equilibrium toward a *greater* concentration in the stationary phase (i.e. larger C_s) and a lower concentration in the mobile or gas phase (i.e. smaller C_m). Therefore, the retention time t_r increases with boiling point.

In any chromatographic separation, one has to select the appropriate separation conditions, including the choice of column type, gas flow rate, temperature, and whether to use a temperature gradient (i.e. a column temperature that changes *during* the separation). For this study, we choose a set of organic compounds, each of which is volatile and thermally stable. We will investigate the GC resolution of the compounds at two different temperatures. The chromatographic separations will be performed at a constant temperature, rather than using a temperature gradient.

Table 1. Properties of Compounds in the Unknown Sample

Compound	Molecular Weight	Chemical Formula	Boiling Point (°C)
Cyclohexane	84.16	C_6H_{12}	80.8
Toluene	92.15	$C_6H_5CH_3$	110.6
Ethyl Benzene	106.17	C_8H_{10}	136.2
para-xylene	106.17	C_8H_{10}	138.4
ortho-xylene	106.17	C_8H_{10}	144.4

For the experiment, you will be given a mixture that contains several volatile compounds whose properties are summarized in Table 1. You will use a capillary column that is 30 m long and has an inner diameter of 0.25 mm, with a stationary phase consisting of a 0.25-μm film of an HP-5 (5%-Diphenyl-95%-Dimethylsiloxane copolymer inner coating) column. You will perform isothermal separation of the mixture at two different temperatures, 80°C and 120°C, both at a gas inlet pressure of 15 psi. The gas for the mobile phase will be H_2 because it supports the flame ionization detector. You will also have samples of the pure compounds that you can inject to help you identify the peaks by their t_r values. You need only run the pure compounds at the lower temperature since we can reasonably assume that the order of their elution will not change at the higher temperature.

Your TA will show you how to set up and start the programmed run and how you know when to inject. Be aware that the temperature change takes a few minutes - the control panel on the gas chromatograph indicates when the column temperature has stabilized and injections can resume. During the change, you should not inject sample until the instrument indicates a stable temperature has been attained, even though the software indicates that it is ready for an injection.

PRE-LAB ASSIGNMENT

Write the answers to these questions in your notebook, and be prepared to show them to your TA at the beginning of the lab period. Be ready to discuss these with your teammates and the TA.

1. Make a simple, neat, hand-drawn hypothetical chromatogram of two closely-spaced GC peaks and use it to explain what is meant by "resolution" in GC or any type of chromatography for that matter. Find an equation in Harris or Skoog that describes how resolution is quantified. Label these parameters in your hand-drawn chromatogram.
2. Draw the chemical structure of all compounds in Table 1. You may use a hand-drawn sketch (then scan it in to your report) or structure-drawing software such as ChemDraw if you have it.
3. Predict which two compounds will be the most difficult to resolve, and explain your logic.
4. Would you expect the chromatographic peaks to come out earlier or later at the high temperature? Explain your reasoning.
5. Would you expect the chromatographic resolution to improve or degrade at the high temperature? Explain your reasoning.

EXPERIMENTAL

Prepare small vials with samples of the individual components and the mixture. Use the HP software to run the preset program for the run. Your TA will demonstrate proper injection technique. It is important to have one person perform all injections to minimize variation in injection technique.

Warning: These compounds are flammable and vapors may be harmful. Use gloves when you handle them. Carefully clean up any spills and properly dispose of any extra solvent.

Record and print out your chromatograms of the individual components at the lower temperature. Record and print out your chromatograms of the mixture at the two temperatures.

SAFETY AND DISPOSAL

Wear gloves when handling the organic solvents and their mixtures, even when injecting the samples.

Dispose of the liquid samples in the organic waste jug in the hood. All vials and caps should be discarded in the chemical trash (the white box).

WRITTEN REPORT

1. Did the compounds elute in order of boiling point (in case of an unexpected result, please explain)? Did an increase in temperature increase or decrease resolution? Was your prediction correct for the two compounds that you expected to be the most difficult to resolve?
2. Use your knowledge of basic thermodynamics to explain why the resolution improved or degraded at the higher temperature.
3. Use Excel to prepare a plot of Eq. 2. Plot K vs t_r for different values of ratios of V_s/V_m and overlay the various curves. Use values of V_s/V_m equal to 10^{-8}, 10^{-4} and 10^{-1}. What do the trends indicate, and why do we use capillary columns?
4. If you wanted to rapidly monitor the levels of these compounds in an environment, and have reasonable accuracy, which temperature would you choose? There is no right or wrong answer, because it depends on how much speed and accuracy you need. But one answer is probably wiser than the other for this particular case. Explain the reasoning behind your choice.