Only very rarely are real-world samples made of a single chemical component. Instead, they usually consist of a few to several hundred different componenents, each varying in concentration from the ultra-trace level up to more than 90%. Such multi-component samples used to be painstakingly separated using a series of chemical and/or physical separation steps such as extraction, distillation, and precipitation. After a significant amount of effort, the sample could then be analyzed by any number of instrumental (e.g. spectroscopy) or chemical (e.g. titrimetric) methods. Even as late as the 1960s and 1970s most analytical methods involved such physical or chemical separation steps.

In the early 20th century, a Russian chemist, Mikhail Tswett, developed a technique for separating a complex plant pigments extract into its individual components in a single step. The technique became known as chromatography (chroma = color) because each of the separated pigments were highly colored. While widely used as a preparative technique, the analytical applications of chromatography did not become commonplace until the 1940s and 1950s, when capillary gas chromatography columns and pressurized liquid chromatography equipment were introduced. The importance of chromatographic these techniques were recognized by the awarding of a Nobel prize to Martin and Synge in 1952 for the development of partition chromatography.

Today, a large percentage of analyses use some form of chromatography to separate a complex mixture into its components for detection by spectroscopic, electrochemical, mass spectrometric, or other types of detectors. The technique has found applications in such diverse applications as space exploration (Mars and Titan probes), homeland security, nutritional analysis, proteomics, drug testing, forensics, environmental testing, industrial process control, and quality assurance. Few scientific techniques have such widespread use and importance to the world's economy.

TEMPERATURE PROGRAMMING AND van DEEMTER CURVES IN GAS CHROMATOGRAPHY

Text reference: Chapter 26, Sections A-F, Chapter 27, Sections A and B

Objectives: The objectives of this experiment are to develop an understanding of how temperature programming affects separations in gas chromatography and to recognize the dependence of chromatographic efficiency on mobile phase linear velocity.

Introduction.

In gas chromatography, a volatile solute partitions between the gaseous mobile phase and the stationary phase (usually a liquid, but occasionally a solid). The partitioning expression can be written as:

$$A_{mobile} \rightleftharpoons A_{stationary}$$

or

$$A_{gas} \rightleftharpoons A_{liquid}$$

At a constant separation temperature (an *isothermal* separation), species with lower boiling points (or higher vapor pressures) will reside more in the gas phase than in the stationary phase and will be swept out the column quickly. Thus, compounds with low boiling points will elute sooner than species with high boiling points. In order to separate a mixture containing compounds with a wide range of boiling points, *temperature programming* is usually

employed. Low boiling point species are first eluted at a low temperature, and then the separation temperature is increased so that the higher boiling point species will also elute.

The quality, or *efficiency*, of a chromatographic separation is measured in theoretical plates, *N*. A large number of theoretical plates corresponds to a high separation efficiency. The number of theoretical plates can be calculated from the retention time, t_R, and peak width at the base, *w*, or at half height, $w_{½}$:

$$N = 16\left(\frac{t_R}{w}\right)^2 = 5.54\left(\frac{t_R}{w_{½}}\right)^2 \tag{23}$$

Note that units of t_R and *w* (or $w_{½}$) must be the same.

Efficiency can also be expressed by the height of a theoretical plate, *H*, which is obtained by dividing the column length by the number of theoretical plates:

$$H = \frac{L}{N} \tag{24}$$

From these definitions, it should be apparent that individual determinations of *N* and *H* must be made for each compound, and that conditions affecting t_R or w (or $w_{½}$) will also affect *N* and *H*.

One condition that affects the separation efficiency is the rate of mobile phase flow through the column. This is an easily-adjusted experimental parameter that can dramatically affect the separation efficiency. This is described by the van Deemter expression, which can be expressed in a simple, general form as:

$$H = A + \frac{B}{u} + Cu = A + \frac{B}{u} + (C_S + C_M)u \tag{25}$$

where *u* is the mobile phase linear velocity[4] and *A*, *B*, *C*, C_S, and C_M are coefficients related to various processes occurring in the chromatographic column (see Ch. 26 for a more detailed discussion of these coefficients). The van Deemter equation predicts that chromatographic efficiency is low (or *H* is large) at very low ***and*** very large values of *u*. At an intermediate flow rate, *H* reaches a minimum and therefore the separation efficiency is maximum. This optimal mobile phase linear velocity, where efficiency is the greatest, is often given the name u_{opt}.

In this experiment, you will be provided with a mixture of straight-chain alkanes having a wide difference in boiling points. You will investigate the effect of temperature on the isothermal elution of the species (at low and high temperatures) and then develop a temperature program for separating all of the compounds with good resolution in less than 10 minutes. Next, you will inject a 2 or 3-component mixture at various the inlet pressures (which gives different linear velocities, *u*) to investigate flow dependence of the separation efficicency.

Procedure.

- **Print out a report for all chromatograms. Be sure to note the conditions (i.e. the temperature program) on the printouts.**

[4] *u* is determined by dividing the column length by the time it takes for an unretained compound to travel through the column. In this experiment, this is the time between injection and the appearance of the solvent (hexane) at the detector.

A solution containing a series of straight-chain saturated hydrocarbons dissolved in hexane will be provided in the lab. You will be provided with a list of the hydrocarbons in the solution. Use the *CRC Handbook of Chemistry and Physics* available in the lab to look up the boiling points and molecular masses of each compound.

1. Isothermal Elution

Load the method "315_PROG.MET" and set the oven temperature to 30° below the lowest boiling point component in the mixture. When the oven is equilibrated at the new temperature, inject 0.5 – 1 μL of hexane. This injection will serve as the blank and will help you to recognize the solvent peak in the chromatogram. You may stop the run after the large solvent peak elutes.

Next, inject 0.5 – 1 μL of the hydrocarbon mixture. ***Do not end the run early.*** After this run is complete (10 min), set the oven temperature to the highest boiling point component of the mixture and set the run time to 5 minutes. ***(Do not set the temperature above 300°C, as the stationary phase in the column will degrade.)*** Watch the detector signal as the temperature increases to the new value. ***After 5 minutes*** inject 0.5 – 1 μL of the mixture at the higher temperature.

- *Did you find as many peaks as expected in the chromatogram recorded at the lower temperature? If not, what happened to the other compounds?*
- *Why was it necessary to wait for a period of time after increasing the temperature for the second injection?*
- *Did you find as many peaks as expected at the higher temperature? If not, what happened to the other compounds?*

2. Temperature Programming

Use the ChemStation software to create a temperature program. Your goal is to find conditions so that the separation can take place in less than 10 minutes and no peaks overlap with the solvent or any other compound. Reasonable starting conditions would be an initial temperature somewhat below (~20 – 30 °C) the lowest boiling point compound and a final temperature equal to the highest boiling point compound.

Adjust the temperature program to accomplish the goal of a 10-minute separation. Realize that you can vary the initial hold time, the rate of the temperature ramp (do not exceed 40°C/min), and the final temperature hold time. Additionally, it is possible to have two different ramps for particularly difficult separations. (This will probably not be necessary!) Finally, there is never a reason to **decrease** the temperature during a separation. **Be sure to record the final temperature program in your lab notebook. Also, print a chromatogram using the optimized temperature program for each group member.**

- *Why is the temperature never decreased during a separation?*

3. van Deemter Curves

A mixture of two or three alkanes in hexane has been prepared for this portion of the lab. Load the method "vanDeem" and inject 0.5 – 1 μL of the mixture using the default conditions. You may stop the run when all peaks have eluted. Next, change the inlet pressure and repeat the injection. Repeat this process 3 more times for a total of 5 chromatograms recorded at different inlet pressures. Inlet pressures can be in the range of about 4 to 40 psi; at least one of your injections must be at a low inlet pressure (< 10 psi). ***Note: If the GC begins to beep when you set the higher pressures, immediately <u>increase</u> the column head pressure on bottom left of the instrument's front panel.***

- *What do you think the value of u_{opt} is?*
- *Which portion of the van Deemter curve for GC did you record? (See Figure 26-7 in book, but don't ignore the values of H and u listed in the figure.)*

Prepare a table listing the compounds in your mixture and their boiling points and molecular masses.

1. Isothermal Elution

Report the two temperatures used for the isothermal elution.

2. Temperature Programming

Report the optimized temperature program. On the chromatogram for this temperature program, sketch (by hand) the temperature program. To do this, add a temperature axis to the right-hand side of the chromatogram and plot the temperature as a function of time. You may wish to use a colored ink to improve the readability of this figure.

3. van Deemter Curves

For the compounds in your mixture (excluding the solvent), construct a van Deemter plot (theoretical plate height as a function of mobile phase linear velocity). It is acceptable to plot data for all of the compounds on the same graph. To determine the linear velocity, divide the column length by the time it takes for the solvent to reach the detector (the time that the solvent peak **starts** to rise). Estimate this time from the chromatogram; do not use the retention time for the solvent listed in the report.