Experiment 17

Determination of Oil-Spill Sources Using Pattern Recognition of Known Petroleum Product Gas Chromatograms

Objectives—There are several objectives to be achieved in this experiment: These include:

1. Introducing you to the basic principles of gas chromatography with thermal conductivity detection

2. Investigating the concept of headspace analysis

3. Observing the pattern of peaks obtained in the gas chromatograms of petroleum and its refined products, and using the patterns to identify the source of environmental hydrocarbons

Introduction—Most common fuels (e.g., gasoline, kerosene, diesel fuel) are mixtures of low-boiling fractions of petroleum. They consist of low molecular weight aliphatic and aromatic hydrocarbons. Solvents, on the other hand, are usually mixtures that may contain aromatics, chlorinated hydrocarbons, aldehydes, and ketones. It is often easy to distinguish between fuels and solvents, but it can be difficult to distinguish between various fuels and between various solvents.

Studies of potential arson cases, hazardous waste incidents, or oil spills require certainty in the results of an analysis, since criminal and civil litigation may result.

The correct interpretation of analyses and the ability to make sound judgments based on qualitative data takes years of experience. Nevertheless, the bases for such judgments are fairly simple. The method of pattern recognition will be used to identify the contaminant, and this experiment will be used to acquaint the student with the requirements of such subjective interpretations.

In this experiment, you are the "expert witness" in the chemical investigation of a spill of an unknown liquid on a beach. A sample of sand containing an organic residue of suspicious origin is taken at the scene. You are asked to analyze the residue. Gas chromatography is used since it is well suited for the analysis of mixtures of volatile organic species. Standard mixtures of several organic chemicals found in fuels and solvents are analyzed to provide chromatograms for comparison. The residue is analyzed by "headspace analysis" to determine the type of organic material present. For safety, the sample is obtained from the residue at room temperature. If the sample is old, or contains high-boiling components, the sample container can be heated by immersing it in hot water to provide more vapor in the headspace; however, the samples used will be sufficiently concentrated in the gaseous phase so they will not require heating. Comparing the chromatograms for known standards with that for the unknown should permit the identification of the fuel or solvent contained in the sample.

A set protocol must be strictly followed so that the retention indices and response factors obtained in the analysis of the standards can be reproduced. Following injection of standards of known composition, samples of the volatile fraction of the residue will be injected into the gas chromatograph.

Theory—Gas chromatography is an instrumental method used to separate a mixture of volatile compounds into its various components. Although it is excellent for separating complex mixtures, it is not good at identifying specific compounds.

The basic components of a gas chromatograph are shown in Figure 17-1. The injection port is used to introduce the sample into the chromatographic system. The column is where the separation of the mixture actually occurs. After eluting from the column, the sample components are carried (via a gas called the "carrier gas") into the detector where they are sensed. Finally, the readout responds to the electrical signal produced by the detector and produces peaks, the areas of which are proportional to the amount of that component in the mixture.

Figure 17-1 Components of a Gas Chromatograph
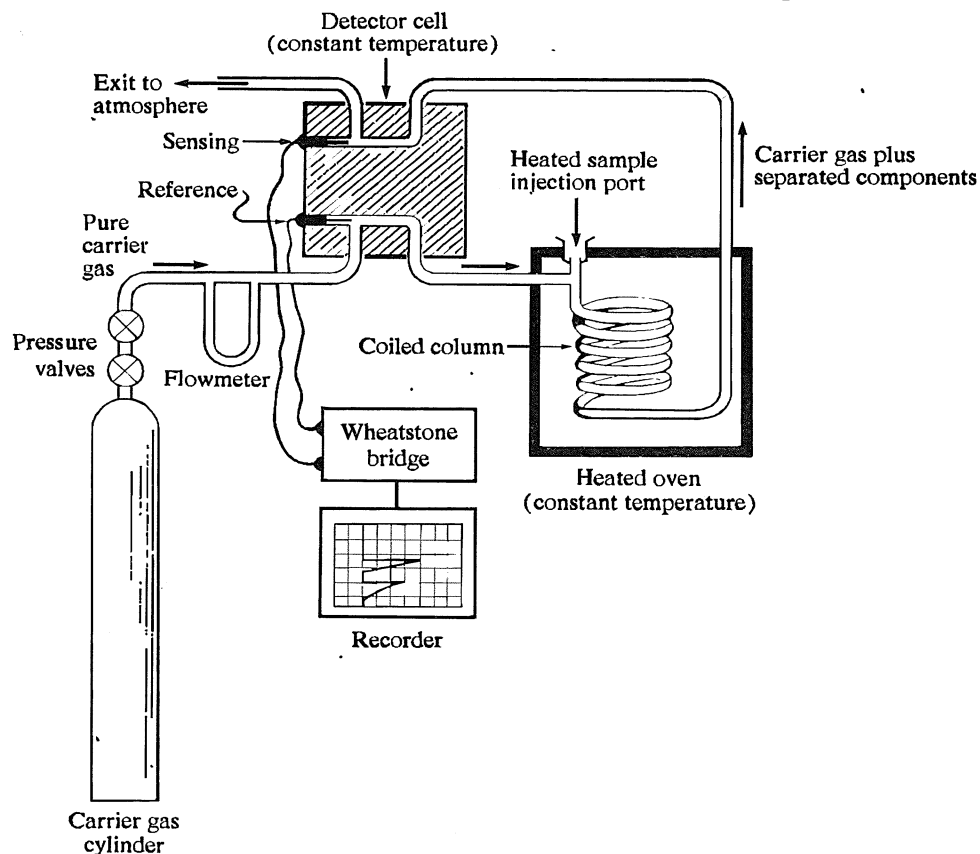
Source: G. H. Schenk, R. B. Hahn and A. V. Hartkopf, *Introduction to Analytical Chemistry*, 2nd ed., Allyn and Bacon, Boston, 1981, p. 424. By permission.

The Injection Port This is where the sample (usually liquid) is introduced into the chromatographic system. The injection is usually done with a microliter syringe that penetrates a rubber-type septum, which provides an airtight system. The temperature of the injection port can be adjusted and is usually held at a temperature such that all the components of the mixture are flash-vaporized when injected.

The Column After the sample is injected, it is carried into the column where the separation of the mixture occurs. There are a number of column types, but we shall examine just one type, a packed column. A packed column is filled with a porous, granular support that is covered with a uniform coating of a nonvolatile liquid, **stationary phase.** The solid support is chosen to have a large surface area, which is needed to support an ample quantity of the coating.

The basis of gas chromatography is the partitioning of components of the mixture between two phases, usually liquid and gaseous phases. The theory for gas-liquid chromatography is very similar to the theory for solvent-solvent extraction as discussed in Experiment 14.

The nature of the stationary phase is chosen to have the same electrical character as the components of the mixture to be separated. If a mixture of hydrocarbons is to be separated, the stationary phase chosen is nonpolar. On the other hand, a mixture of low molecular weight alcohols is better separated if a more polar and hydrogen-bonding phase is employed.

Gas chromatographic separations depend on the different rates at which the components of a sample travel through the column. If two substances, Y and Z, have the same affinity for the liquid phase, but Z is more volatile than Y (that is, has a lower boiling point), then Z will remain in the gaseous phase longer as it travels down the column than does Y. Thus, Y and Z are separated on the basis of their boiling points. In general, substances are separated on the basis of their boiling points if the polarities of the compounds are about the same. Table 17-1 lists the boiling points of simple alkanes and can be used to predict the order of elution in a gas chromatographic separation.

The column is enclosed in an oven used to maintain a constant temperature or for changing the temperature.

The Detector Several types of detector are used in gas chromatography. The simplest and one of the most widely used types is the thermal conductivity detector (TCD). This type of detector uses a Wheatstone bridge circuit to detect the sample components as they exit to the detector. The carrier gas most often used in gas chromatography is helium, although nitrogen can also be used. Helium is preferred as it has a very high thermal conductivity that allows more sensitive detection of the components. Organic compounds have smaller thermal conductivities than helium, and when a compound elutes at the detector, the temperature of the detector filament increases. This sensor is one arm of a Wheatstone bridge circuit. The final result is an imbalance in the bridge that causes a flow of electricity to a recorder that responds to the current (or voltage).

Results of a Gas Chromatographic Analysis There are two important results of a chromatographic analysis: the time it takes for a particular compound to elute to the detector, the **retention time,** and the **peak area.** The retention time, R_t is a function of the column temperature, the flow rate of the carrier gas, the column length, the nature of the stationary phase, and the physical properties of the analyte. Generally, the chromatographic identification of a compound is made by determining the retention times for a series of

standards (usually in a mixture, such as a series of *n*-alkanes) and then measuring the retention times for each compound in the unknown mixture. Then if the unknown mixture gives peaks that have identical retention times as the standards, it is assumed that those peaks are due to the same compound as in the known mixture. Unfortunately, just because two peaks have the same retention time does not guarantee that they are the same compound. Verification is frequently required, and can be accomplished by using a mass spectrometer coupled to the gas chromatograph (called a GC/MS). This technique is widely used for environmental analysis, drug testing and many other areas.

The peak area of a component of a mixture is proportional to the mass of that component in the mixture. Thus, the peak area can be used to calculate the relative amount of that component in the mixture. If peaks are sharp, they are almost triangular in shape and the area can be calculated using the product of one-half the base length times the height of the triangle. The total area is calculated and the percentage of any one peak component is its area times 100 divided by the total area.

**Table 17-1** Boiling Points of Some Simple Hydrocarbons

Name	Boiling Point (°C)	Name	Boiling Point (°C)
Butane	-0.5	2,2,3-Trimethylpentane	109.8
Pentane	36	2,3,3-Trimethylpentane	114.8
Hexane	68	2,2,4-Trimethylpentane	99.2
Heptane	98.4	2-Methylheptane	118
Octane	125.6	3-Methylheptane	115–118
Nonane	150.8	4-Methylheptane	118
Decane	174.1	2,3-Dimethylheptane	141
Dodecane	216.3	2,4-Dimethylheptane	133
2-Methylbutane	27.9	2,5-Dimethylheptane	136
2,2-Dimethylbutane	49.7	2,6-Dimethylheptane	135
2,3-Dimethylbutane	58	3,3-Dimethylheptane	137
2-Methylpentane	60.3	4-Ethylheptane	141
3-Methylpentane	63.3	2-Methyloctane	142.8
2,2-Dimethylpentane	79.2	3-Methyloctane	144-145
2,3-Dimethylpentane	89.8	4-Methyloctane	142.4
3,3-Dimethylpentane	138.2	2,7-Dimethyloctane	159.6
2-Methylhexane	90	Cyclohexane	81
3-Methylhexane	92	Benzene	80.1
2,2-Dimethylhexane	107	Toluene	110.6
2,3-Dimethylhexane	116	*o*-Xylene	144
2,4-Dimethylhexane	111	*m*-Xylene	139
2,5-Dimethylhexane	109	*p*-Xylene	138

Source: R. C. Weast, Editor-in-Chief, *CRC Handbook of Chemistry and Physics*, 59th ed., CRC Press, Boca Raton, FL, 1979.

Pattern Recognition—Figure 17-2 (top) shows the gas chromatogram of a sample of number 2 fuel oil taken from a ship which ran aground near the mouth of the St. Johns River in Jacksonville, Florida. Figure 17-2 (bottom) shows the gas chromatogram of a sample from a tar ball, found on a beach a few miles from the ship that was extracted for hydrocarbons. Even a cursory examination indicates that the two samples came from the same source. This identification by pattern similarity is called "pattern recognition" and has been used for many years to identify environmental PCBs (they have a very characteristic chromatographic pattern).

Although such identifications can be put on a more quantitative basis, pattern recognition is very useful in environmental studies. It can be made more exact by acquiring an extensive series of gas chromatograms for many different sample types, such as a series of petroleum-based products, and then comparing a sample suspected of being one of these types with the "library," which was assembled from chromatograms of known sample types.

Figure 17-2 Gas Chromatograms of (top) Number 2 Fuel Oil Taken from a Ship that
ran Aground off Jacksonville, Florida, and (bottom) Hydrocarbons
from a Tar Ball Found Several Miles from the Oil Spill
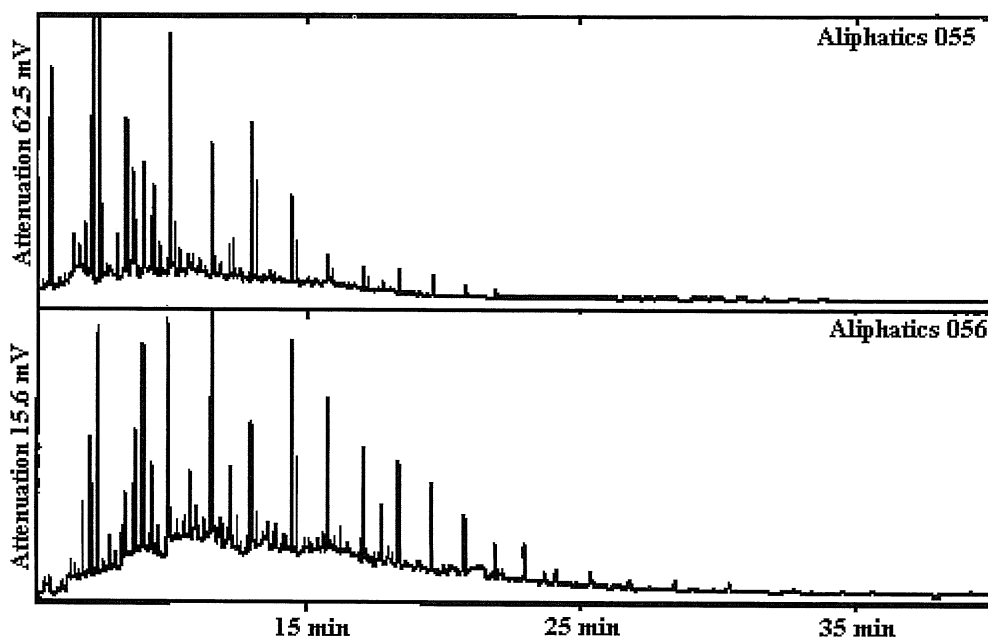
In this experiment, you will prepare a "mini-library" of reference chromatograms. These will be the chromatograms of the headspace vapors of different petroleum-derived sample types. Then the chromatogram of the unknown will be determined and by visually comparing the chromatograms, the nature of the unknown can be determined.

You will also determine the chromatogram of a series of simple alkanes and another of low molecular weight alcohols. Because retention times are linearly related to their boiling points, the retention times will be characteristic of each compound. Then by comparing retention times of the standards with those in the petroleum-derived samples, some of the compounds in the unknowns can be identified.

One potentially confusing point is that standard alkanes and alcohols will be injected in liquid form in the microliter volume range. The sand (or soil) samples will be examined for compounds in the vapor phase at room temperature. Since liquids are about 1000 times more dense than the vapor, a sample of at least 1 mL of vapor must be injected to obtain a comparable signal. Fortunately, the sensitivity range of the thermal conductivity detector is wide enough to sense small concentrations. Based on comparing the R_t values of the standards with the unknowns, you will get a more quantitative "feel" for the identity of your contaminant.

Safety Precautions

1. Safety glasses must be worn at all times in the chemistry laboratory.

2. Do not stand in front of a reduction gauge on a gas cylinder when opening the cylinder. There is a danger of its rupturing when opening the main valve.

3. The injection port of the gas chromatograph will be very hot. Be careful not to touch it.

Procedure—A mixture of *n*-alkanes, from pentane through nonane, and one of alcohols, from methanol through butanol, are used to obtain chromatograms. The mixtures have been prepared using different, and known, quantities of each component. One objective of this experiment is for you to determine the percentage of each component in each mixture using peak areas from the chromatograms.

Second, the headspace vapors from each reference petroleum-derived mixture and the unknown, will be injected into the gas chromatograph and their gas chromatograms obtained.

Injection of the Liquid Samples

Table 17-2 gives suggested injection volumes, but the actual volume injected depends on the sensitivity of your chromatographic system. To avoid overloading the column, it is best to inject small volumes and increase the sample size if necessary. It saves time if several students work together to obtain the chromatograms and then share copies. However, each student should inject at least three samples. Inject the following samples and obtain the chromatograms.

1. Inject 2–3 μL of the alcohol mixture and obtain the chromatogram. Thoroughly rinse the syringe with pentane by drawing pentane into the syringe and plunging it out into a waste container. This should be repeated at least five times to thoroughly clean the syringe.

2. Repeat Step 1 using the mixture of *n*-alkanes.

The conditions for a typical chromatographic analysis of alkanes are given in Table 17-2.

Table 17-2 Chromatographic Conditions for the Separation of Alkanes

Chromatographic Variables	Typical Value(s)
Detector current	150 mA
Column(Choice)	4' length x ¼ in. diameter; 20% Carbowax
	20M or DC200 on Chrom P(80–100 Mesh)
Column temperature	110°C
Flow rate	40–45 mL/min
Recorder scale	10 mV
Chart speed	2.5 cm/min
Attenuation	2(or as needed)
Injection volume, liquid	2–3 μL
Injection volume, vapor	1 mL

Headspace Analysis

1. Erlenmeyer flasks containing soil or sand have been treated with samples of gasoline, kerosene, jet fuel, shellac thinner, and mineral spirits. Each flask contains a different sample, and the tops of the flasks are sealed with septa. Do not remove the septa as volatile components in the gaseous phase may be lost, which will alter the composition of the head space gases.

2. Each student selects a known sample, which is identified, and withdraws a 1 mL sample of the headspace gases. This sample is then injected into the gas chromatograph. The syringe must be gas-tight. The gas chromatogram is then obtained. The collection of chromatograms for the known samples is the "mini-library." This procedure is repeated until all the known samples have been injected.

3. Each student is then given an unknown sample to identify. The chromatogram is obtained in the same way as for the known samples.

Waste Minimization and Disposal

1. If the normal alkanes and alcohols are in containers with rubber septa, they are stable indefinitely and do not have to be disposed of. If the alkane and alcohol mixtures need to be disposed of, they are turned over to a commercial hazardous waste contractor.

2. The samples of gasoline, kerosene, etc. in sand or soil are hazardous wastes and can be combined and disposed of by a commercial facility. Alternatively, the samples can be used for a period of years if additional sample is added to the matrix.

Data Analysis

1. For the standard alcohol and alkane mixtures, write the retention times above each peak on the gas chromatogram. If the peaks are triangular, estimate the areas by multiplying one-half the base length times its height. If peaks are not sharp, estimate the areas by counting the number of squares under each peak on the chart paper. The percentage composition of a particular component is its area divided by the total area times 100. Calculate the percentage composition of each component in each mixture.

2. Prepare a table of retention times for each component and its percentage in the mixture.

3. Prepare two plots, one for each standard mixture, of retention time (ordinate) versus the boiling point of the compound. Carry out a least-squares analysis and obtain the correlation coefficient.

4. Describe the pattern of chromatographic peaks for the unknown sample for which a headspace analysis was performed. Which pattern for the known samples most closely resembles this pattern? The early peaks are often the most relevant for this comparison since they have the most reproducible retention times.

5. Gas chromatograms for liquid samples of gasoline, kerosene, etc., will be available for comparisons.

Supplemental Activity

1. A wide variety of petroleum-derived products can be examined in this experiment. Samples of high volatility are preferable since higher-boiling components will not elute from the column.

2. To have more time with the gas chromatograph, one of the more important tools in environmental science, the student can be allowed to determine the effect on the chromatogram of the *n*-alkanes by changing the following: flow rate, column temperature, and sample size.

3. It would be informative to warm the headspace samples by about 10°C and obtain a new chromatogram to see the effect of the increased temperature.

Questions and Further Thoughts

1. Which mixture separates better (i.e., has the greater distance between peaks), the alcohols or the alkanes? Explain the difference.

2. Which alcohols are present in shellac thinner? Are alcohols present in any of the other samples?

3. Which alkanes are present in gasoline, and which are present in the other samples?

4. Use the data of Table 17-1 to plot boiling point (ordinate) versus carbon number for the *n*-alkanes. Discuss the linearity with respect to the intermolecular forces. Is there a relationship between R_t and the boiling points? Make tentative boiling-point assignments for the peaks observed in the headspace of your soil sample. Assuming it is a hydrocarbon, give a tentative assignment for each peak.

Notes

1. Many terms are important in the field of gas chromatography, such as solid phase, absorbent, eluent, relative retention time, support, etc. It may be helpful for the student to compile a "mini-dictionary" of these "buzzwords," using several literature sources.

2. It is important that the gas chromatograph be ready when students begin this experiment. It takes over an hour for the thermal conductivity (TC) detector to stabilize.

3. When the detector current is on there must be a flow of carrier gas through the detector. Otherwise, the detector will be destroyed.

Literature Cited

1. A. P. Bentz, Oil spill identification, *Anal. Chem.*48(6):454A–472A (1976).

2. D. L. Duewer, B. R. Kowalski, and T. F. Schatzki, Source identification of oil spills by pattern recognition analysis of natural elemental composition, *Anal. Chem.,*47(9):1573–1583 (1975).

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4. R. C. Weast, Editor-in-Chief, *CRC Handbook of Chemistry and Physics,* 59th ed., CRC Press, Boca Raton, FL, 1979.