Experiment 18

Analysis of Environmental Hydrocarbons Using Simple Extraction and Analysis by Flame-Ionization Detection Gas Chromatography

Objective—The objective of this experiment is to introduce you to one of the most important analysis methods used in environmental chemistry, flame-ionization detection gas chromatography (FID/GC). You will also learn how to extract trace amounts of hydrocarbons as well as the higher levels that would be found in an oil spill.

Introduction— Hydrocarbons are particularly important pollutants because of the large volumes of petroleum transported, spillages in transfer, and the impact from sewage outfalls and stormwater runoff. Small quantities of oils and greases can contaminate large quantities of water, and the aromatic components can be harmful to shellfish and finfish. Many aromatic compounds and their metabolites are potent carcinogens.

Petroleum, or crude oil, is a mixture of hundreds of compounds, most of which are hydrocarbons (HCs). Hydrocarbons are compounds of carbon and hydrogen and are structurally classified as aliphatic or aromatic. The aliphatic HCs may be straight-chain (called normal) or branched. Aliphatic HCs include the alkanes and cycloalkanes, which are illustrated in Figure 18-1. Aromatic HCs are characterized by a ring structure with alternate single and double bonds as shown in Figure 18-2. Some monocyclic aromatic HCs associated with pollution from gasoline include benzene, toluene, ethylbenzene, and the xylenes.

Fused ring systems, shown in Figure 18-3, are called polycyclic aromatic hydrocarbons (PAHs) or polynuclear aromatic hydrocarbons (PNAs), some of which are the most carcinogenic substances known.

Hydrocarbons found in the environment are mainly of petrogenic origin. However, low levels are ubiquitous in nature. Elevated levels are usually associated with human activities and thus are termed anthropogenic hydrocarbons. This term usually refers to refined products obtained from petroleum.

Petroleum refining begins with the separation of crude oil into various fractions by fractional distillation. Several important fractions are shown in Table 18-1. The origin of HCs in the environment is given in Table 18-2.

The Effects of Environmental Petroleum

Fish exposed to petroleum in water, sediments, and food supply rapidly take up HCs which accumulate in tissues of the liver, brain, and muscle.

The predominant chemical change in petroleum on entering the environment is oxidation. Oxidation occurs either through photochemical reactions or enzymatic reactions in microorganisms. Hydrocarbons that

dissolve in water quickly evaporate or become diluted and are ultimately flushed from the stream.

Since petroleum HCs are hydrophobic, and thus lipid soluble, a major transport mechanism is via their association with suspended particulates. Sediments are major reservoirs (sinks) for such pollutants.

Microbial degradation is the most important process involved in the weathering of petroleum in the environment, and the rate decreases in the following order: *n*-alkanes > branched alkanes > aromatics > cycloalkanes.

Figure18-1 Structures of Aliphatic Hydrocarbons

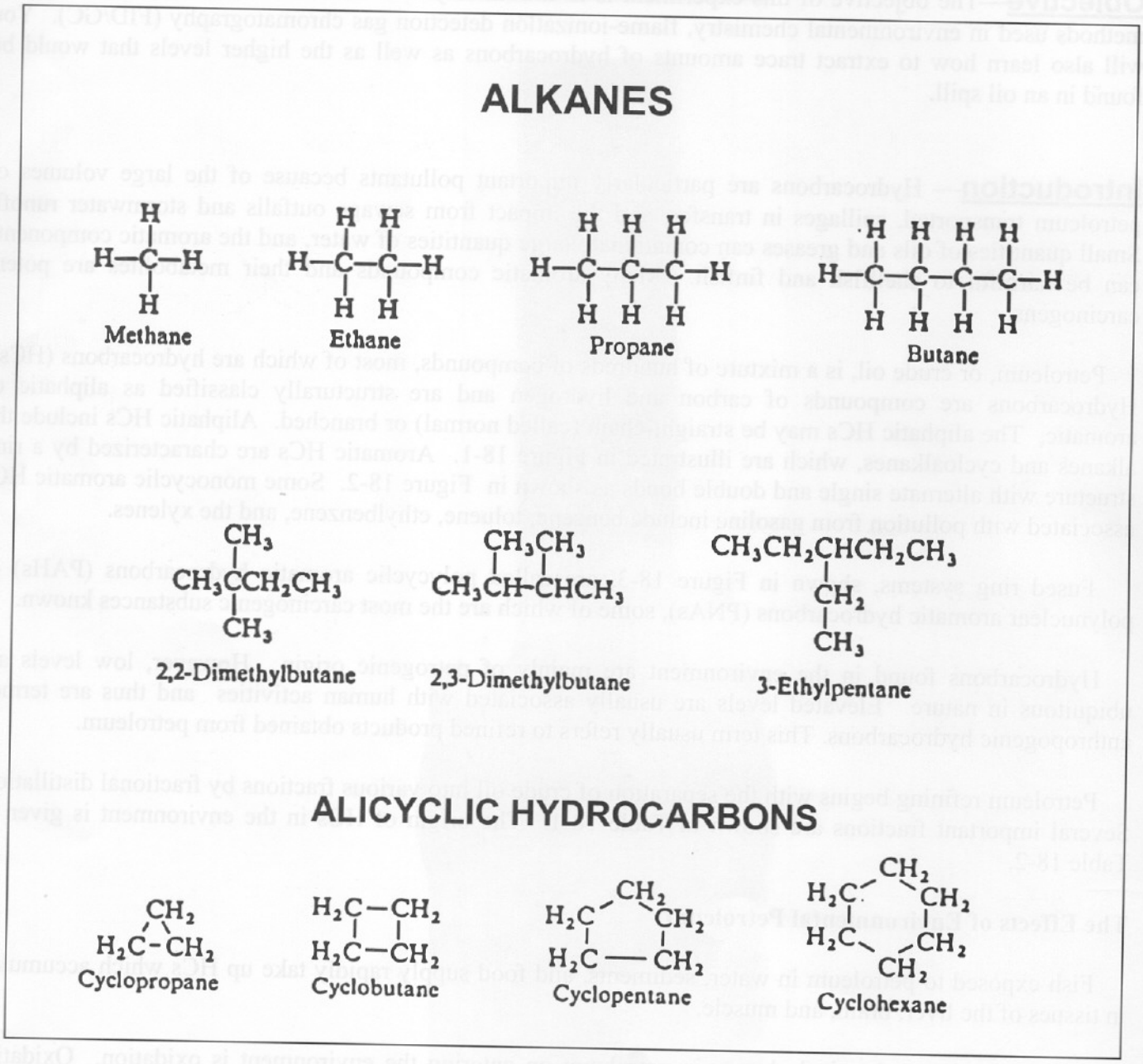


Figure 18-2 The Simplest Aromatic System, Benzene

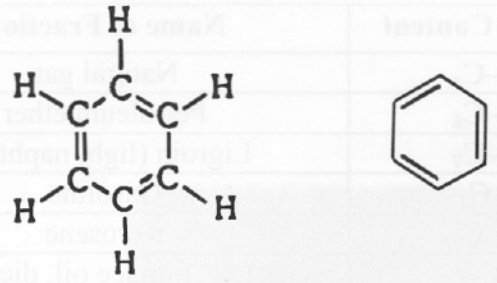


Figure 18-3 Polycyclic Aromatic Hydrocarbons

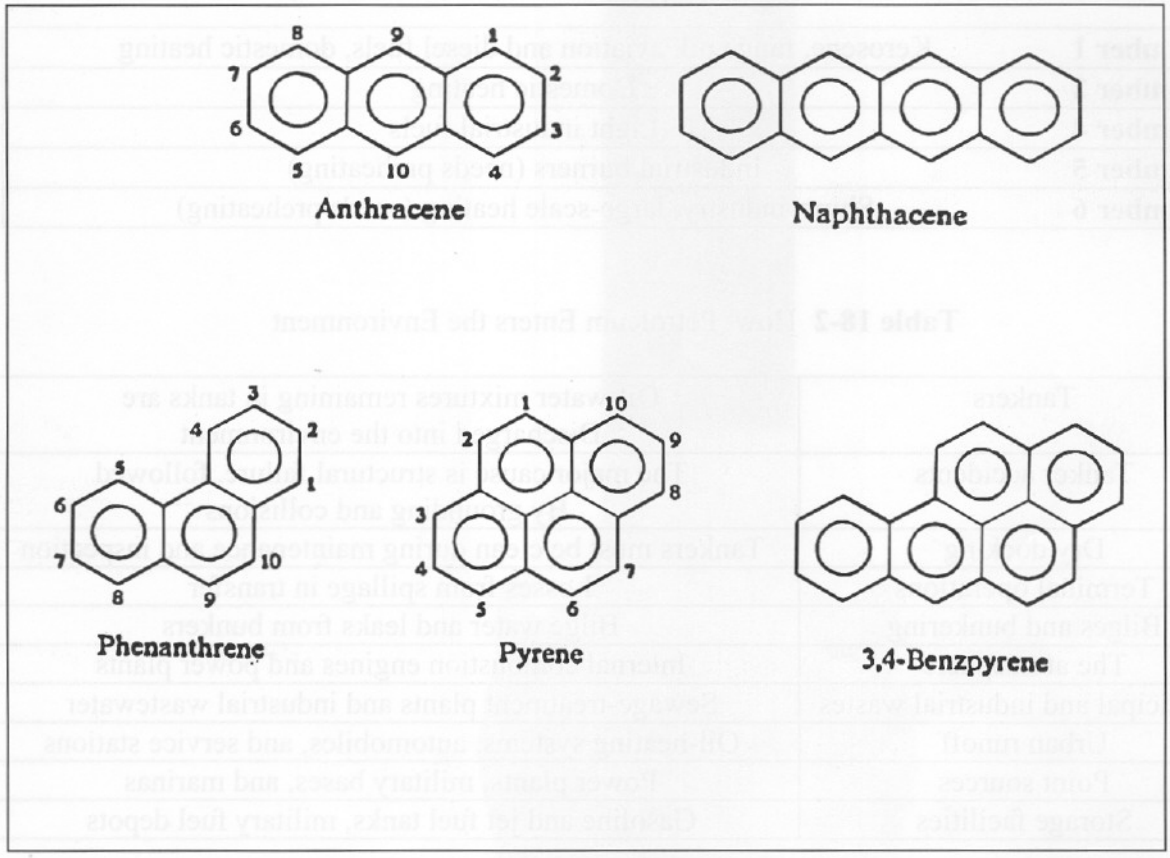


Table 18-1 Substances Obtained by the Refining of Petroleum

Carbon Content	Name of Fraction
C_1–C_4	Natural gas
C_5–C_6	Petroleum ether
C_6–C_7	Ligroin (light naphtha)
C_6–C_{12}	Gasoline
C_{12}–C_{18}	Kerosene
> C_{18}	Gas Oil (furnace oil, diesel oils)
> C_{18}	Lubricating oils
> C_{18}	Waxes
> C_{18}	Asphalt

Fuel Oils

Number 1	Kerosene, range oil, aviation and diesel fuels, domestic heating
Number 2	Domestic heating
Number 4	Light industrial fuels
Number 5	Industrial burners (needs preheating)
Number 6	Ships, industry, large-scale heating (needs preheating)

Table 18-2 How Petroleum Enters the Environment

Tankers	Oil-water mixtures remaining in tanks are Discharged into the environment
Tanker accidents	The major cause is structural failure, followed By grounding and collisions
Dry docking	Tankers must be clean during maintenance and inspection
Terminal operations	Losses from spillage in transfer
Bilges and bunkering	Bilge water and leaks from bunkers
The atmosphere	Internal combustion engines and power plants
Municipal and industrial wastes	Sewage-treatment plants and industrial wastewater
Urban runoff	Oil-heating systems, automobiles, and service stations
Point sources	Power plants, military bases, and marinas
Storage facilities	Gasoline and jet fuel tanks, military fuel depots

Theory—The HCs most readily identifiable by gas chromatography are the normal alkanes. These HCs are separated on the basis of their boiling points, the lower boiling point compounds elute from the column first and the least volatile ones elute at a later time. Figure 18-4a shows the gas chromatogram of an environmental sample that illustrates the spacings of the *n*-alkane peaks from a relatively clean environment. Figure 18-4b shows the chromatogram of the HCs from a contaminated, but unweathered,

environment. The hump is characteristic of petrogenic HCs. Figure 18-4c shows the chromatogram of a weathered environmental sample of petrogenic HCs. The normal alkanes have been largely biodegraded, leaving unresolved cycloalkanes in the hump.

Figure 18-4a Gas Chromatogram of a Relatively Clean Environmental Sample Showing the Peak Spacings of the *n*-Alkanes

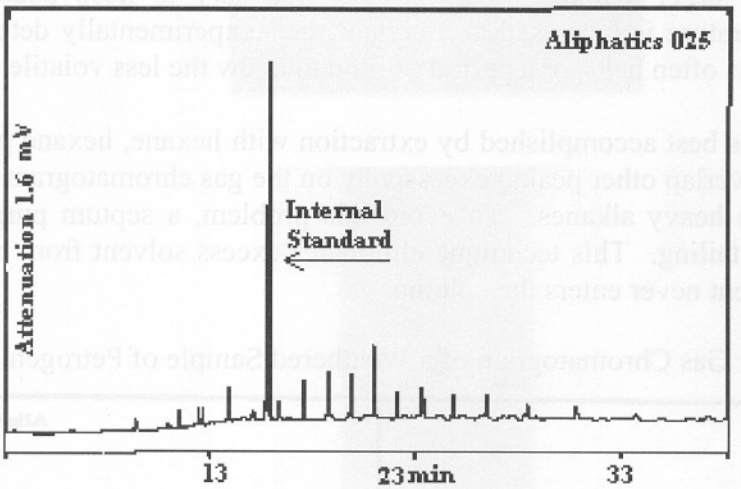
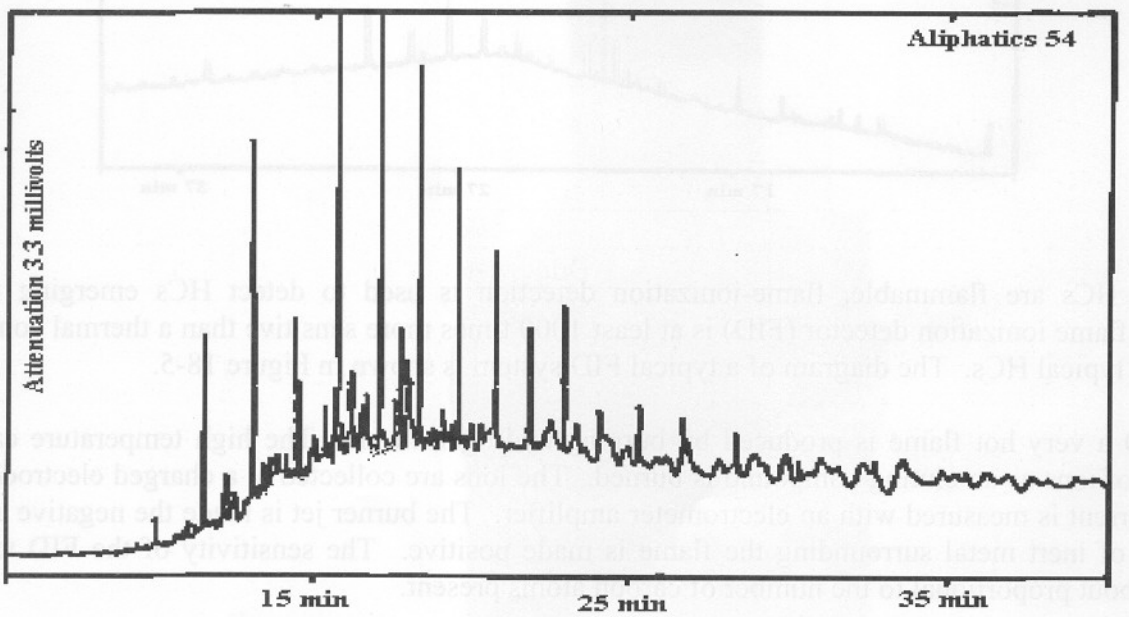


Figure 18-4b Gas Chromatogram of the Alkanes from a Contaminated Unweathered Sample of Petrogenic Hydrocarbons

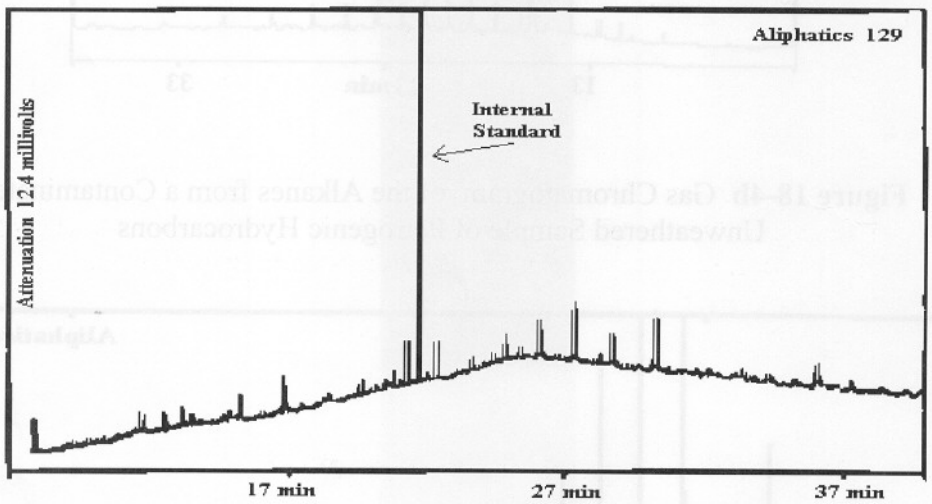


Recently contaminated sediment samples often result in gas chromatograms with alkane peaks ranging from fewer than 10 to about 30 carbon atoms. Generally, however, alkanes with fewer than about 12 carbon atoms are too volatile to persist in the environment for any length of time. Being hydrophobic, if there is no suspended silt onto which the HCs can sorb, they will tend to migrate to the surface where hydrophobic interactions are less, and at the surface they can rapidly evaporate.

Due to the great range of alkanes usually present, temperature programming is necessary to decrease retention times for the larger alkanes. This is also necessary to keep peaks sharp. In temperature programming the temperature is increased at a certain rate (experimentally determined) until it reaches a maximum value, which is often held for a period of time to allow the less volatile components to elute.

Since isolating HCs is best accomplished by extraction with hexane, hexane predominates in the extract and would tail (that is, overlap other peaks) excessively on the gas chromatogram and thus interfere with the quantitation of even the heavy alkanes. To avoid this problem, a septum purge is done after injection, eliminating most of the tailing. This technique eliminates excess solvent from the region near the septum and thus the excess solvent never enters the column.

Figure 18-4c Gas Chromatogram of a Weathered Sample of Petrogenic Hydrocarbons

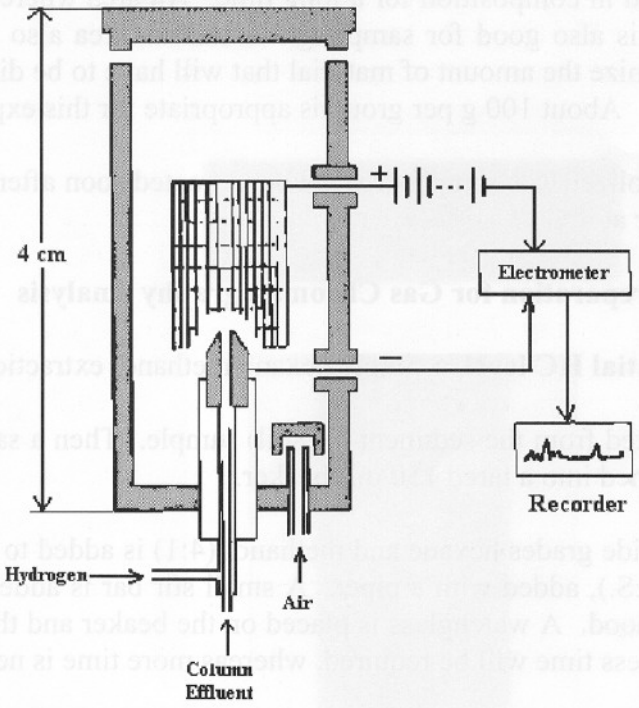


Because HCs are flammable, flame-ionization detection is used to detect HCs emerging from the column. A flame ionization detector (FID) is at least 1000 times more sensitive than a thermal conductivity detector for typical HCs. The diagram of a typical FID system is shown in Figure 18-5.

In a FID a very hot flame is produced by burning hydrogen in air. The high temperature causes the production of ions as an eluting compound is burned. The ions are collected at a charged electrode and the resulting current is measured with an electrometer amplifier. The burner jet is made the negative electrode, and a loop of inert metal surrounding the flame is made positive. The sensitivity of the FID to organic species is about proportional to the number of carbon atoms present.

Quantitation of the gas chromatogram can be done manually using peak heights, but it is better to use a computerized data-handling system, preferably one that can be used to calculate results and change the attenuation after the chromatographic run.

Figure 18-5 The Flame Ionization Detector



Source: G. W. Ewing, *Instrumental Methods of Chemical Analysis*, 5th ed., McGraw-Hill, Inc., New York, 1985, p. 360. By permission.

Safety Issues

1. Safety glasses must be worn during all parts of this experiment.
2. The instructor must be present when the gas chromatograph is being used.
3. Use gloves when handling aromatic substances.
4. Avoid physical contact with toluene and also avoid breathing its vapor.

Procedure

A: Sample Collection and Storage

Avoid using plastic devices for sample collection or storage. Plastics contain plasticizers that can interfere with the analysis. In fact, if sediment samples are collected from an urban stream, plasticizers will frequently be extracted with the HCs, and they often dominate the gas chromatogram.

Water samples are not easily studied for HCs since HCs are only slightly soluble in water. An area where there has been an oil spill that has washed up onto a sandy beach is ideal for sample collection. Tar ball samples remain unchanged in composition for a long time. An area where there has been considerable fuel transportation or storage is also good for sampling. A marina area also provides excellent sediment samples for analysis. To minimize the amount of material that will have to be disposed of eventually, collect a minimum amount of sample. About 100 g per group is appropriate for this experiment.

Since many bacteria metabolize HCs, samples should be extracted soon after collection. Otherwise, they must be stored in a refrigerator at 4°C.

B: Sample Extraction and Preparation for Gas Chromatography Analysis

If the sample has a **substantial HC level**, a simple hexane/methanol extraction will suffice.

1. Excess water is first decanted from the sediment (or soil) sample. Then a sample with mass of about 10 g (weighed to 0.1 mg) is weighed into a tared 150 mL beaker.

2. A 50 mL solution of pesticide grades hexane and methanol (4:1) is added to the beaker followed by 0.25 mL of the internal standard (I.S.), added with a pipet. A small stir bar is added and the beaker is put on a magnetic stir plate in a fume hood. A watchglass is placed on the beaker and the sample is stirred for about 0.5 hour. For a sandy matrix less time will be required, whereas more time is needed for a clay-like matrix.

3. After extraction, the sample is gravity filtered (medium porosity paper, Whatman Number 1, for example) into a 150 mL beaker. The extraction beaker and funnel are rinsed with two 5 mL portions of hexane.

4. The combined filtrate and rinsings are dried with anhydrous sodium sulfate, using a magnetic stir bar to stir. The sample is then filtered into a 150 mL beaker, rinsing the previous beaker and funnel with two 5 mL portions of hexane.

5. At this point, nitrogen gas could be used to evaporate excess solvent, or a concentrator, such as a Kuderna-Danish, could be used. However, it is convenient and much less expensive to evaporate the solvent in a fume hood for 6–12 hours. An aluminum tent over the beaker prevents contamination.

6. The sides of the beaker are occasionally rinsed down with hexane, and the evaporation is continued until the final volume is 1 to 2 mL. The sample is now ready for gas chromatography.

An alternative extraction procedure uses the Soxhlet apparatus described in Experiment 14. The Soxhlet procedure is important for the extraction of **very low levels of HCs** but requires much more time and equipment.

C: The Internal Standard

The I.S. solution is prepared by weighing out 10 mg of androstane in a small beaker, dissolving in hexane, and then quantitatively transferring to a 100 mL volumetric flask. The solution is diluted to the mark with hexane and then thoroughly stirred.

If the final volume of the extract before chromatography is about 2 mL, the I.S. concentration will be about 10 ppm if the I.S. volume used is 0.25 mL. Relatively clean environmental sediments have a total HC level less than 10 ppm, whereas very contaminated samples may have total HC levels several orders of magnitude greater. The I.S. may be provided.

D: Gas Chromatography Analysis

Any gas chromatographic system that provides an FID, temperature programming, and computerized data handling can be used. The following instructions apply to a Perkin-Elmer Sigma 3B gas chromatograph with a microprocessor to adjust injector and detector temperatures as well as carry out temperature programming. Instructions will be similar for other instruments.

A DB-5 fused silica capillary column, 0.25 mm in diameter and 30 m in length, gives excellent peak resolution. A constant split of about l0 mL/min is used to maintain a steady flow of gas away from the septum area to prevent back diffusion. Thirty seconds after injection, the septum purge valve is opened to sweep away solvent that continues to desorb. The purge prevents severe tailing of the solvent peak. A purge rate of 4–5 mL/min is used.

An analysis consists of a 2.0–3.0 µL sample injection, the initial temperature of 100°C held for 0 minutes. This is followed by an 8°/min temperature ramp to a final temperature of 280°C. If only lighter HCs are present, this temperature needs to be held for only 5 minutes, followed by automatic cooling back to 100°C.

1. A 10 µL syringe is rinsed at least five times with pesticide grade hexane. It is then rinsed at least two times with the alkane standards. The rinsings are put into a waste vial that is provided.

2. The syringe is filled to 2–3 µL with the standards, any excess sample being injected into the waste vial. The sample is then injected into the gas chromatograph quickly and smoothly. The start button on the instrument is quickly pressed. After 30 seconds the septum purge is opened. **This is a critical step** as it prevents tailing of the hexane peak!

3. The instrument will now produce a gas chromatogram and a report giving the retention times and areas of each peak and additional results if it has been instructed to do so.

4. The syringe is rinsed at least 10 times with hexane.

5. The syringe is then rinsed twice with the environmental HC extract, and then filled with 2–3 µL of extract.

6. The sample is injected into the gas chromatograph, as before. Again open the septum purge after 30 seconds. (The exact time is not critical, but should be between 25 and 35 seconds.)

7. The instrument and data station generate a chromatogram and report as before, but there should be many more peaks on the chromatogram than the previous sample.

E: Data Handling

The gas chromatogram is quantitated by first determining the area corresponding to the I.S. The concentrations of the *n*-alkanes can be calculated by using their areas and assuming a response factor (R.F.) of unity for each. For equal masses the response factor compares the area of a component with the area of a reference peak, such as the internal standard, which is defined as having a response factor of unity. A response factor of 0.85 for a component means that its area is 0.85 times the area for the internal standard if the masses of both are equal in the mixture.

For a HC analysis, an accurate integration of peak areas is required and it is necessary to have the capability of handling a large number of peaks. A Perkin-Elmer Sigma 3600 Data Station is one of several systems that fills the requirements. This device gives the area for each peak and by using the I.S. concentration and the original sample mass, it converts areas into concentrations.

The standard alkane mixture is chromatographed to determine retention times for individual alkanes. The alkanes from dodecane to eicosane will be present in the standard mixture. If time is a factor, a copy of the chromatogram for the standard alkane mixture will be provided.

An option in this experiment is to use the known masses of the alkanes in the standard mixture, and the corresponding areas to calculate response factors for the alkanes. However, these are often close to unity. It is also straightforward to calculate the percentage composition of each alkane and compare the results with the given composition.

After the standard alkane chromatogram has been obtained, the retention time for each component can be entered into the data station software so that when the environmental sample is run, the data station will identify the alkanes and report their areas and the ppm of each present in the mixture.

F: Optional Separation of Aliphatics and Aromatics

An option for this experiment is to separate the aromatic and aliphatic portions using gravity-column chromatography. Since usually only small amounts of HCs are present, a very small column can be used for the separation and the experimental time is short.

1. Hexane is used to add a slurry of silica gel to fill a small column (5 cm or less) to about 40% of its capacity. This is followed with a hexane slurry of alumina, filling an additional 40 percent of the column. A thin layer of clean sand is placed on top of the alumina.

2. The sample is placed at the top of the column and is eluted with three column volumes of hexane. The hexane is then replaced with toluene and the column is eluted with three column volumes of eluent. Evaporation, as previously described, to 2 mL is then followed by gas chromatography. In this option, *ortho*-terphenyl is used as the I.S. for the aromatic fraction. (Use 0.10 mL of 10 mg *ortho*-terphenyl dissolved in 100 mL of hexane, added before the initial extraction with hexane.)

3. If a sample is particularly heavy in HCs, it is possible to quantitate the masses of the two fractions by completely evaporating the solvents. Otherwise, they can be quantitated by using the I.S.

Waste Minimization and Disposal

1. Since interesting samples are contaminated with petroleum hydrocarbons, collect only a minimum amount of sediment or soil since extra material must be disposed of as hazardous waste. The extracted samples must also be treated as hazardous wastes as well and are transferred to a bottle for storage. This will later be given to a hazardous waste contractor.

2. The *n*-alkane standards and the two internal standards, androstane and *ortho*-terphenyl, should be kept in containers with rubber septa where they can be kept for an indefinite period of time, and used in future experiments.

3. The alumina and silica gel (if used) and sodium sulfate are slightly contaminated with hydrocarbons and must be considered to be hazardous wastes. These three substances can be combined in a bottle for later disposal by a waste contractor.

4. Any waste hexane and methanol must be stored in separate bottles for later commercial disposal. Waste toluene should be kept in a separate bottle for later commercial disposal.

Data Analysis

1. Give full sampling details.

2. Submit your chromatogram(s) and the report(s) generated by the data station. Describe the pattern of aliphatic peaks and whether or not there is a hump in the chromatogram. Try to decide if the HCs in the sample are fresh or weathered.

3. Calculate the percentage of each alkane in the standards mixture. Compare with the given results that come with the standards. (The standards may be purchased or may be made up by the lab instructor.)

4. Use the report generated by the data station for the environmental sample to obtain the sum of the ppm of the alkanes of odd numbers of carbon atoms and do the same for the alkanes with an even number of carbon atoms. The carbon preference index (C.P.I.), is the ratio of odd to even hydrocarbons. Find this ratio for your sample. A large C.P.I indicates HCs of biogenic origin. Petrogenic HCs, on the other hand show little preference for either odd or even HCs and the C.P.I. is closer to unity. Also, a longer *n*-alkane range is found with petrogenic HCs; natural HCs often have a narrow range of *n*-alkanes.

5. Report the total HC area and the area for the I.S. Use the amount of I.S. and its area to calculate the total ppm of HCs present in the sample.

6. If the HCs were separated into aliphatic and aromatic fractions, determine the ratio of the two, gravimetrically (if this was done) and by using the internal standards to quantitate the two fractions (if this was done).

7. Discuss the extent of HC contamination of the sample.

Supplemental Activity

1. Instead of environmental samples, samples of petroleum or petroleum products can be studied. Samples of petroleum from different parts of the United States give very different gas chromatograms as do samples from different parts of the world.

2. If aliphatics and aromatics were separated, the aromatics fraction can be examined by fluorimetry as in Experiment 15.

Questions and Further Thoughts

1. Benzene has been used to separate aliphatics and aromatics, but this practice is discouraged because benzene exposure is known to cause leukemia. The disadvantage of toluene is its lower volatility.

2. Some environmental samples have aliphatic hydrocarbons with more than 30 carbon atoms. In this case the hold time at the higher temperature must be increased to purge these heavier hydrocarbons from the column. If this is not done, these compounds will elute with the next injected sample. If broad peaks appear at the start of a new chromatographic run, this is a likely source of the peaks.

Notes

1. Do not exceed the maximum temperature recommended for the column. Higher temperatures can cause the stationary phase to elute or decompose, thus ruining the column—a costly mistake!

2. A new column must be conditioned by allowing carrier gas to pass through it at an elevated temperature (above the temperature at which it will be used) for several hours. When conditioning the column, it must not be connected to the detector or the detector may be harmed!

Literature Cited

1. D. N. Boehnke, C. A. Boehnke, K. I. Miller, J. S. Robertson, and A.Q. White, *Final Report, Survey of Hydrocarbons in the Lower St. Johns River in Jacksonville, Florida*, prepared for the Florida Department of Natural Resources, Tallahassee, FL, October 1983.

2. M. Grayson, Executive Ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Wiley Interscience, Ney York, 1982: Vol. 11, pp. 317-333, 652-695; Vol. 17, pp. 110-131, 183-190, 199-256, 257-271.

3. S. E. Hamilton, T. S. Bates, and J. D. Cline, Sources and transport of hydrocarbons in the Green-Duwamish River, Washington, *Environ. Sci. Technol. 18*(2): 72–79, (1984).

4. D. MacLeod, G. Prohaska, D. G. Gennero, and D. W. Brown, Interlaboratory comparisons of selected trace hydrocarbons from marine sediments, *Anal.Chem., 54*:389–392 (1982).

5. J. E. Ravan, *Regional Coastal Pollution Control,* B. L. Edge, ed., Coastal Zone Pollution Management, Proc. of the Symposium, Clemson University, Clemson, SC, 1972.

6. E. S. Van Vleet and J. G. Quinn, Input and fate of petroleum hydrocarbons entering the Providence River and Upper Narragansett Bay from wastewater effluents, *Environ. Sci. Technol., 11(12):1086–1092,*(1977).