# Experiment 15

Fluorimetric Determination of Polycyclic Aromatic Hydrocarbons

Objective—The objective of this experiment is to learn the use of an extremely sensitive method for quantitatively determining polycyclic aromatic hydrocarbons (PAHs). Also, the relative response of three aromatic hydrocarbons will be measured.

Introduction—Earlier in these experiments we used analytical methods based on the absorption of electromagnetic radiation, namely colorimetry and atomic absorption. We now use a method based on the emission of radiation, fluorimetry. This method can be extremely sensitive for certain types of compounds, detecting less than ppm levels, but very insensitive for other types. Also, it is capable of detecting one of many substances present in a mixture without interference.

Theory—The term <u>fluorescence</u> refers to the emission of radiant energy by a molecule or ion in an excited state. The particle reaches the excited state by absorbing radiant energy, usually from the ultraviolet region of the spectrum. The process may be represented by the scheme shown in Figure 15-1.

Figure 15-1 Diagram of Stepwise Process of Fluorescence
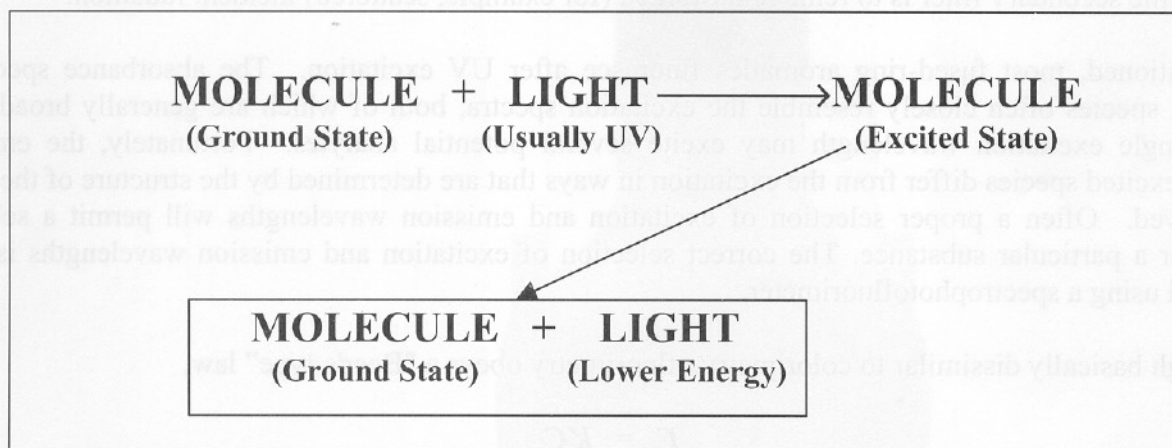
After excitation, the species quickly returns to the lowest vibrational sublevel of the first excited electronic state. The energy lost in this process is transferred to solvent molecules and may ultimately appear as heat.

Fluorescence occurs when a species from the ground vibrational level of the excited electronic state emits electromagnetic radiation. If the species becomes deactivated and returns to the ground state by another method, fluorescence is not observed. Deactivation can occur through collisions with molecules of

solvent or other fluorescing species, resulting in decreased vibrational energy and finally a transition to the ground electronic state. The more flexible a molecule or ion, the easier it is for collisional deactivation to occur. Thus, in most cases only rigid species will fluoresce.

Most substances that fluoresce strongly enough to be useful analytically are organic rather than inorganic. In general, substances that absorb strongly in the UV, usually at wavelengths longer than 250 nm, may fluoresce. Substances that absorb at shorter wavelengths are often subject to photodecomposition rather than fluorescence because of the high energy of the radiation absorbed.

Many aromatic compounds absorb strongly in the 250–320 nm region. Generally, the substances most likely to fluoresce are those with cyclic structures containing conjugated double bonds. Most polynuclear aromatic hydrocarbons fluoresce strongly. The presence of $-OH$, $-OR$, or $-NH_2$ groups increase fluorescence intensity. (R is an organic substituent, such as methyl, ethyl, phenyl, etc.) Groups such as $-COOH$, $-NO_2$, or $-SO_3H$ reduce or even eliminate fluorescence. Ring closure in a compound, as in the formation of a metal chelate, enhances fluorescence.

Different chemical species have characteristic excitation and fluorescence spectra. In order to measure fluorescence, it is necessary to have a source that emits UV radiation, a detector that responds to UV and visible radiation, and a means for separating the excitation and fluorescence radiation.

The experimental arrangement for fluorimetry is shown in Figure 15-2. The excitation radiation is produced by a high-pressure xenon lamp that produces light over a wide range of wavelengths. This radiation then passes through a narrow-bandpass primary filter to give the proper energy range for exciting the species under study. As the sample fluoresces (in all directions) the emitted radiation is measured at right angles to the excitation beam by the detector after it passes through a sharp-cut secondary filter. The purpose of the secondary filter is to remove unwanted (for example, scattered) incident radiation.

As mentioned, most fused-ring aromatics fluoresce after UV excitation. The absorbance spectra of fluorescent species often closely resemble the excitation spectra, both of which are generally broad band. Thus, a single excitation wavelength may excite several potential analytes. Fortunately, the emission spectra of excited species differ from the excitation in ways that are determined by the structure of the molecule involved. Often a proper selection of excitation and emission wavelengths will permit a selective analysis for a particular substance. The correct selection of excitation and emission wavelengths is often determined using a spectrophotofluorimeter.

Although basically dissimilar to colorimetry, fluorimetry obeys a "Beer's-type" law,

$$F = k'C$$

<div align="right">(15-1)</div>

where F is the intensity of emitted fluorescence, k' is a constant that depends on several factors, and C is the concentration. As with colorimetry, a calibration plot is prepared using a series of standard solutions and measuring their fluorescence intensity.

Figure15-2 Diagram of a Filter Fluorescence Photometer
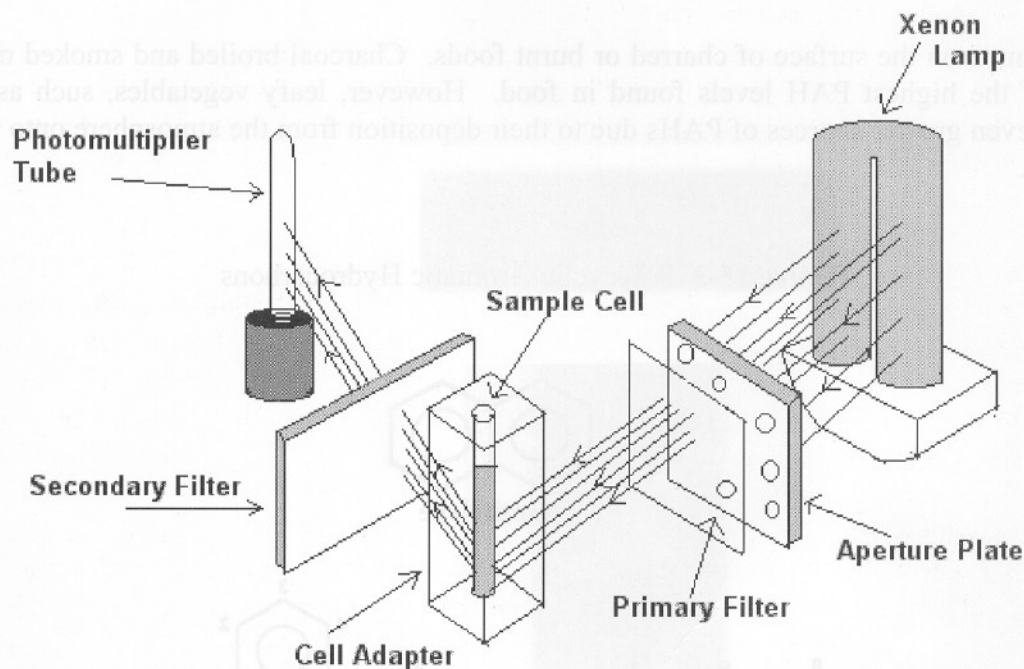
Polycyclic Aromatic Hydrocarbons One of the most useful applications of fluorescence is the determination of aromatic hydrocarbons. Since aromatic hydrocarbons are among the chief air pollutants, the fluorimeter has been used by the Public Health Service for determining these substances. The measurement of the very low concentrations of PAHs found in samples of particulates collected from the atmosphere is among the usual applications.

Aromatic compounds are characterized by a ring structure having alternating single and double bonds, and benzene is the simplest example. Benzene itself fluoresces only slightly due to its vibrational flexibility. When a second ring is attached to the first to give napthalene, which is quite rigid, there is intense fluorescence. A third ring can be attached to the previous ring system in two ways, one giving a linear arrangement called anthracene, and a third nonlinear arrangement called phenanthrene. These substances are illustrated in Figure 15-3. Compounds with fused rings are called polynuclear aromatic hydrocarbons (PNAs) or polycyclic aromatic hydrocarbons. They have a planar geometry that gives them a rigidity, which results in intense fluorescence.

PAHs originate from both biogenic (natural) and anthropogenic (human) sources. The incomplete combustion of hydrocarbons is the chief means of PAH formation. Among the PAHs found in urban air are naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)pyrene, benzo(*a*)anthracene chrysene, and many others.

PAHs are present at low levels in automobile exhaust. Higher PAH levels occur when large amounts of soot are also present, as in diesel exhaust or smoke from coal or wood fires. Atmospheric PAHs are found almost exclusively in the solid phase, usually sorbed to soot particles. PAHs are produced from saturated hydrocarbons at high temperatures under oxygen-deficient conditions. Elevated PAH levels are likely to be

encountered in polluted urban air and in the vicinity of forest fires. Coal furnace stack gas may contain 1000 µg/m^3 PAHs.

PAHs are formed on the surface of charred or burnt foods. Charcoal broiled and smoked meat and fish contain some of the highest PAH levels found in food. However, leafy vegetables, such as lettuce and spinach, can be even greater sources of PAHs due to their deposition from the atmosphere onto the leaves of these vegetables.

Figure 15-3 Polycyclic Aromatic Hydrocarbons

Napthalene

Anthracene   **Phenanthrene**
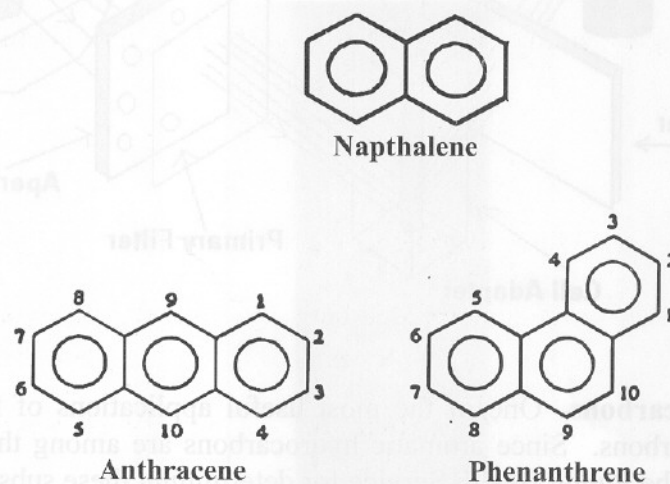
The chemical composition of tobacco smoke is complex and contains thousands of components, many of which are carcinogens. Environmental tobacco smoke consists of gases and particulates. The gases include CO, NO$_2$, HCHO, PAHs, and other volatile organic compounds (VOCs). The particulate phase is called <u>tar</u>, which contains nicotine and the less volatile hydrocarbons.

Cigarette smoke contains about 100 µg/m^3 PAHs. The concentration of toxic products of partial combustion is actually higher in second-hand smoke than in mainstream smoke since combustion occurs at a lower temperature in a smoldering cigarette than in one through which air is inhaled.

The presence of naturally occurring PAHs is an interesting feature of soil organic matter. PAHs found in soil include fluoranthene, pyrene, and chrysene. In rivers and lakes, anthracene and phenanthrene are found attached to sediments rather than dissolved in the water. PAHs containing four or fewer rings usually exist in the gaseous state when released into the atmosphere. After less than one day they are degraded by free radical reactions since PAHs are light-sensitive.

Compounds with four or more benzene rings fused together can be potent carcinogens. Their cancer-causing capacity is due to activation by the same class of liver enzymes that metabolizes toluene and other xenobiotics. When these enzymes add oxygen to the PAHs, they produce epoxide adducts that interact

strongly with the bases of DNA to alter genes. The PAHs that are the most potent carcinogens possess a <u>bay region</u> formed by a branching of the benzene ring sequence.

PAHs enter the aquatic environment as a result of oil spills from tankers, refineries, and offshore drilling. Also, the leaching of PAHs from the creosote used to preserve the immersed lumber of fishing docks represents a significant source of pollution to crustaceans such as lobsters. PAHs have been found to bioaccumulate in the fatty tissues of some marine organisms, and have been linked to the occurrence of liver lesions and tumors in some fish.

High levels of benzo(a)pyrene have been found in the sediments at several urban sites in the Great Lakes. It bioaccumulates in the food chain (log K_{ow} = 6.3; see Experiment 16). Methyl-substituted PAHs are often more potent carcinogens than the parent hydrocarbons.

The PAH levels in drinking water are usually less than a few ng/L, an insignificant level for humans.

Safety Issues

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

2. Wear gloves when handling aromatic hydrocarbons. Be careful to avoid skin contact with these substances.

3. Do not pour any unused reagents down the drain, but dispose of wastes according to your instructor's directions.

Procedure

1. **Preparation of Calibration Curve:** A 1.0 x 10^{-3} M stock solution of anthracene (17.8 mg/100 mL cyclohexane) will be provided. Transfer 1.0 mL of the stock solution to 10, 25, 50, and 100 mL volumetric flasks and dilute to the mark with cyclohexane. Use cyclohexane as the blank, and determine the fluorescence intensity of each standard using the operating procedure for your instrument.

2. Prepare 100 mL of 1 x 10^{-2} M solutions of toluene, naphthalene, and biphenyl using cyclohexane as solvent. Measure the fluorescence intensity of each solution.

3. Extract a used cigarette filter by placing it in a beaker with about 25 mL of ethanol (the ethanol provides both hydrophilic and hydrophobic interactions to extract the hydrocarbons in an aqueous environment). Stir for 0.5 hour. Dilute 1 mL of the extract with enough ethanol to give 25 mL of solution. Measure the fluorescence intensity of this solution. If the fluorescence intensity is not in range, adjust the dilution appropriately.

Waste Minimization and Disposal

1. A considerable volume of hazardous waste can be generated in this experiment. Efforts should be made to minimize the wastes. The calibrating solutions of anthracene in cyclohexane can be shared to minimize the wastes due to this source.

2. The toluene, naphthalene, and biphenyl solutions can be combined and put into a bottle for later commercial disposal. The solution of anthracene in cyclohexane can also be combined with the other aromatic wastes.

3. The alcohol extract of a cigarette filter should be put into a designated container for later disposal by a commercial hazardous waste facility.

Data Analysis

1. Prepare a calibration curve of fluorescence signal (as ordinate) versus concentration of anthracene. Compare with the plot shown in Figure 15-4. Estimate the lowest concentration of anthracene that could be accurately measured by fluorimetry. How does it compare with UV spectroscopy? The curvature in Figure 15-4 is due to self-absorbance by the anthracene when it is present at high concentrations.

2. For the test samples, prepare a table of fluorescence intensity and concentration, as determined from the calibration plot.

3. Compare the fluorescence intensity of toluene, naphthalene, and biphenyl. Discuss the effect of structure on fluorescence intensity. Is it valid to use anthracene to estimate other PAHs?

4. Calculate the "effective anthracene concentration" for the cigarette filter extract. Estimate the mg of anthracene in the filter, assuming that the fluorescence is due entirely to anthracene.

Supplemental Activity

1. If the previous experiment has been completed and a sample of environmental hydrocarbons has been extracted, this may be diluted in cyclohexane and its fluorescent intensity measured to give an estimate of PAHs present in the sample.

2. In a previous experiment the supplemental activity suggests separating aliphatic and aromatic hydrocarbons. This can be carried out for many sample types, including crude oils, or a Soxhlet-extracted sample of oils and greases. This sample can also be quantitated for PAHs.

3. Another important application of fluorimetry is the determination of the concentration of spent sulfite liquor in water. References to this and other applications are given in the Literature section.

4. One of the later experiments in this manual includes the collection of particulates from the atmosphere. These can be extracted for PAHs and then analyzed by fluorimetry.

Figure 15-4   Anthracene Calibration Curve
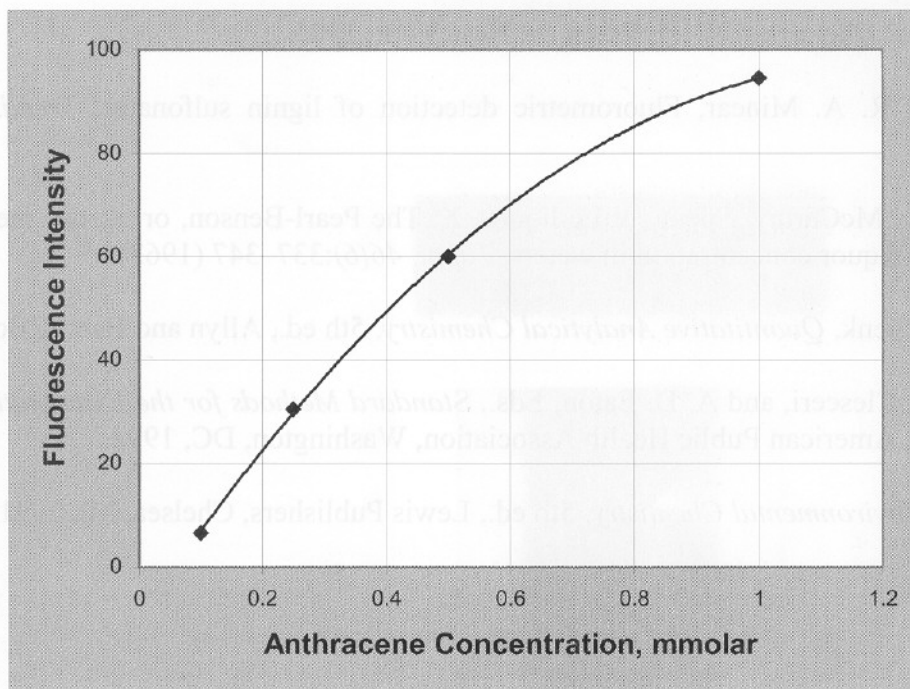
Questions and Further Thoughts

1. Devise a study to show how the difference in the PAH content of second-hand smoke from a cigarette and inhaled smoke could be detected.

2. What are PAHs exposed to in the atmosphere that causes their destruction?

3. Lichens frequently contain fluorescing compounds, and these can be extracted and the total fluorescence intensity determined. Lichens, themselves, are very sensitive to certain air pollutants.

Notes

Must find λ for excitation and emission

1. The fluorocolorimeter is a direct-reading filter fluorimeter used for the quantitative determination of substances with known excitation and emission spectra. Prior to using the instrument, the proper filters must be installed. In this experiment, a good choice of primary filter is a PC-6 narrow-pass filter. A PC-9A sharp-cut filter is a good choice for the secondary filter.

2. Before use, the instrument is turned on and allowed to stabilize, the meter is zeroed, and the photometer dark current subtracted from the meter reading. Dark current is a measurable signal present even when there is no light striking the PM tube.

Literature Cited

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