

Separation of Polyaromatic Hydrocarbons Using 2-Dimensional Thin-Layer Chromatography

An Environmental Chemistry Experiment

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Conventional (1-dimensional) thin-layer chromatography (TLC) is taught in most, if not all, undergraduate chemistry courses as being a powerful analytical tool. However, students rarely have the opportunity to use 2-dimensional thin-layer chromatography (2-D TLC). Two-dimensional thin-layer chromatography is touched upon in many laboratory texts (1), but is rarely used as a means of separation and identification and is often viewed as a means for the organic chemist to check if a compound is decomposing on the TLC plate.

We have recently introduced a second-year environmental chemistry course into our curriculum, and it was decided during the planning of the experimental aspect of the course that it would be beneficial for the students if they became familiar with the technique of 2-D TLC. Polyaromatic hydrocarbons (PAHs) were chosen as the "subject" of the 2-D TLC experiment because of their environmental importance as air and soil contaminants in many industrial countries. PAHs are also commonly detected by gas-liquid chromatography and high-performance liquid chromatography (2). We decided to combine the 2-D TLC analysis with a Soxhlet extraction of

a PAH-containing soil sample; in this way, students become familiar with the Soxhlet extraction method as well as with 2-D TLC.

Each group of three students is given an "unknown" soil sample containing two different PAHs, from which they extract previously added PAHs. While this extraction is proceeding, they run the 2-dimensional thin-layer chromatogram of a standard mixture of four PAHs. This chromatogram is then compared with that of their soil extract to identify the components in the unknown. R_f values and fluorescence colors (under UV light) are used to obtain definitive results (3). The experiment is, on average, completed in three hours. It is not advisable to complete the extraction and then analyze the extract in a different lab session, as the PAHs tend to decompose in solution.

The solvent systems and type of TLC plate we adopted for the 2-dimensional thin-layer chromatography are taken from a reported analysis of PAHs in water (4).

The experiment was designed to be able to be performed by a relatively large group of students (ca. 30) at one time. To this end, we used two standard solutions, each containing four components:

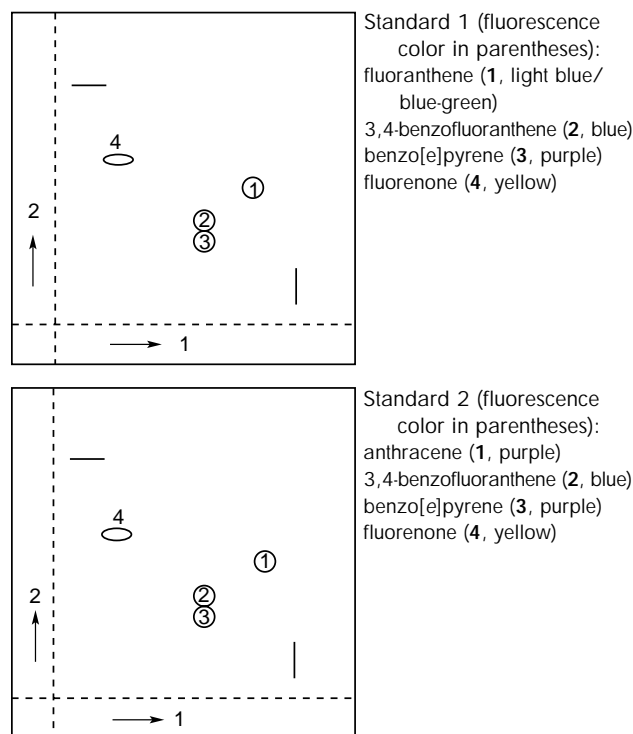


Figure 1. Components and 2-dimensional thin-layer chromatograms of standards 1 and 2.

<i>Standard 1:</i> fluoranthene	<i>Standard 2:</i> anthracene
3,4-benzofluoranthene	3,4-benzofluoranthene
benzo[e]pyrene	benzo[e]pyrene
fluorenone	fluorenone

While three of the PAHs are common to both standard solutions, the fourth component is different. This allowed the preparation of nine different "unknown" soil samples each containing two PAHs. Representative 2-D TLCs of standards 1 and 2 are shown in Figure 1. We found that students often had difficulty in identifying the compounds responsible for the purple spots in standard 2. This provides a good opportunity to discuss how polarity affects R_f values. Where there are no functional groups present, polarity increases with the size of the molecule. Thus, of the two purple fluorescent compounds in standard 2, anthracene will be less polar than benzo(e)pyrene, causing it to travel further up the TLC plate in the first development. The students are also required to search the literature to find the structures of the PAHs present in their standard solution.

Student feedback for the experiment has been positive, and students are appreciative of being able to perform a "real" analysis. The disadvantages to the experiment are that PAHs are highly toxic and, in some cases, expensive. However, we felt that the small amounts used would not pose too great a health risk, and that it was important for students to learn how to handle such toxic materials in a safe and responsible manner.

Experimental Procedure

CAUTION: PAHs are carcinogens. Nitrile gloves should be worn at all times and all operations involving PAHs should be carried out in a fumehood. Ensure all solvent and PAH residues are disposed of in appropriate residue containers.

Soxhlet Extraction

The soil sample (10 g) was continuously extracted with 75 mL of dichloromethane for 45 min to 1 h (6–8 fills of the Soxhlet reservoir). The extract was reduced to approximately half of its original volume (rotary evaporator or steam bath) and analyzed by 2-dimensional thin-layer chromatography.

2-Dimensional Thin-Layer Chromatography

Two intersecting pencil (*not* ink!) lines were ruled 1.5 cm from each edge of a 10 × 10-cm glass-backed TLC plate (Machery-Nagel ALOX/CEL-AC-Mix-25; a mixture of basic aluminium oxide and acetylated cellulose). The solution to be analyzed was spotted at the intersection of the two lines and then developed (protected from daylight) in the first solvent system of hexane/chloroform (98:2 v/v) to a distance of 5 cm from the origin (development time ca. 15 min). The plate was dried thoroughly in air, turned 90°, and developed (protected from daylight) in the second solvent system of methanol/ether/distilled water (4:4:1 v/v) to a distance of 5 cm from the origin (development time ca. 30 min). After drying thoroughly in air, the plate was visualized using 254-nm UV light.

To Be Done before the Class

Preparation of Standard Solutions. One milligram of each component in 1 mL of dichloromethane (keep solution wrapped in foil and store in a refrigerator).

Preparation of Soil Samples. Ten grams of predried soil (or sand) plus 5 mg *each* of two PAHs. Using the two standard solutions, this gives a set of 9 possible unknowns. It is best not to prepare large batches and separate into smaller samples later, as the PAHs tend not to distribute through the soil evenly. Although the preparation of the samples individually is slightly more time consuming, it ensures that the correct amount of each PAH is present in each sample.

Note

^WSupplementary materials for this article (a list of equipment and supplies, notes for the instructor, and a handout with instructions for students) are available on *JCE Online* at <http://jchemed.chem.wisc.edu/Journal/issues/1999/Dec/abs1691.html>.

Literature Cited

1. For example: Harwood, L. M.; Moody, C. J. *Experimental Organic Chemistry*; Blackwell Scientific: Oxford, 1989; pp 166–167.
2. For detection of PAHs using gas–liquid chromatography, see: <http://www.varian.com/inst/csb/gcnotes/gc16.html>. For detection of PAHs in water using GC-MS, see: <http://www.varian.com/inst/csb/gcmsnote/gcms44.html>. For detection of PAHs using high-performance liquid chromatography, see: <http://www.varian.com/inst/csb/hplcnote/lc08.html>. (All sites accessed Sep 1999.)
3. For a recent paper discussing fluorescence detection in relation to TLC, see: Cawley, J. J. *J. Chem. Educ.* **1995**, *72*, 272.
4. Kunte, H.; Borneff, J. *Z. Wasser-Abwasser-Forsch.* **1976**, *9*, 35.