

## Environmental Chemistry II - Experiment 8

*Each group of 3 students will need -*

1 x Soxhlet extraction apparatus (approx. 40 ml capacity)  
1 x cellulose Soxhlet thimble to fit (22 x 80 mm)  
1 x 100 ml **or** 125 ml round bottom flask (to fit Soxhlet lower joint)  
1 x double surface condenser (preferable to fit Soxhlet upper joint, but an adapter may be required)

Boiling chips

Heating mantle to fit round bottom flask

2 x developing chambers to fit a 10x10 cm tlc plate (1 L beakers may be used for this)

2 x 10x10 cm alumina/ acetylated cellulose tlc plates

2 x covers for developing chambers (petri dishes or watch glasses)

2 x 100 ml measuring cylinders

2 x 100 ml conical flasks

aluminium foil

tlc spotters

condenser tubing

dichloromethane

hexane

chloroform

methanol

diethyl ether

distilled water

PAH-containing soil sample

*Students will also need access to -*

rotary evaporator / steam bath

254 nm UV lamps

previously prepared PAH standard solutions

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### *Recipe for unknown soil samples*

10 g of pre-dried soil (or sand) + 5 mg **each** of **two** unknown PAH's

It is best not to do large batches and separate them into smaller samples later, as the PAH's tend not to distribute through the soil evenly. Although preparation of individual samples is slightly more time-consuming, it ensures that the correct amount of each PAH is present.

Demonstrator notes contain Unknown numbers and their components.

### *Recipe for standard solutions*

1 mg of each component in 1 ml of dichloromethane (keep solution wrapped in foil and store in a refrigerator).

Demonstrator notes contain the required components for the two standard solutions.

### *Tlc plates*

The plates used are Machery/Nagel glass backed plates, coated with a mixture of basic aluminium oxide and acetylated cellulose (product name ALOX/CEL-AC-Mix-25; catalogue number 810 053). We obtained the plates from Selby Biolab Scientific, Australia.

The plates come as 20x20 cm, and need to be cut into four 10x10 cm plates. The only thing that needs to be made sure of when doing this is that the plates fit comfortably into the developing chambers. If trimming is required, try to avoid doing it on the factory-cut edge, as this provides a nice even edge for developing.

Preparation of all nine unknown soil samples and the two standard solutions will take one person less than an hour. Cutting the TLC plates to the required size is more time-consuming; however, enough plates for a class of 30 students can be cut in less than an hour.

### *CAS Numbers*

anthracene	[120-12-7]
fluoranthene	[206-44-0]
3,4-benzofluoranthene	[205-99-2]
benzo(e)pyrene	[192-97-2]
fluorenone	[486-25-9]
dichloromethane	[75-09-2]
hexane	[110-54-3]
chloroform	[67-66-3]
methanol	[67-56-1]
diethyl ether	[60-29-7]

## Experiment 8

### Identification of Polyaromatic Hydrocarbons in Soil samples using 2-Dimensional Thin Layer Chromatography

Conventional (1-dimensional) thin layer chromatography (TLC) is covered in the Chemistry I course. It is a powerful analytical tool, but sometimes compounds cannot be separated satisfactorily in a given solvent system. This is where 2-dimensional TLC (2-D TLC) can be of use. In 2-D TLC, the plate is developed twice in two different directions and in two different solvent systems (hence, 2-D). The plate is developed in one solvent system, dried, then turned 90° and developed in the second solvent system (see Figure 1).

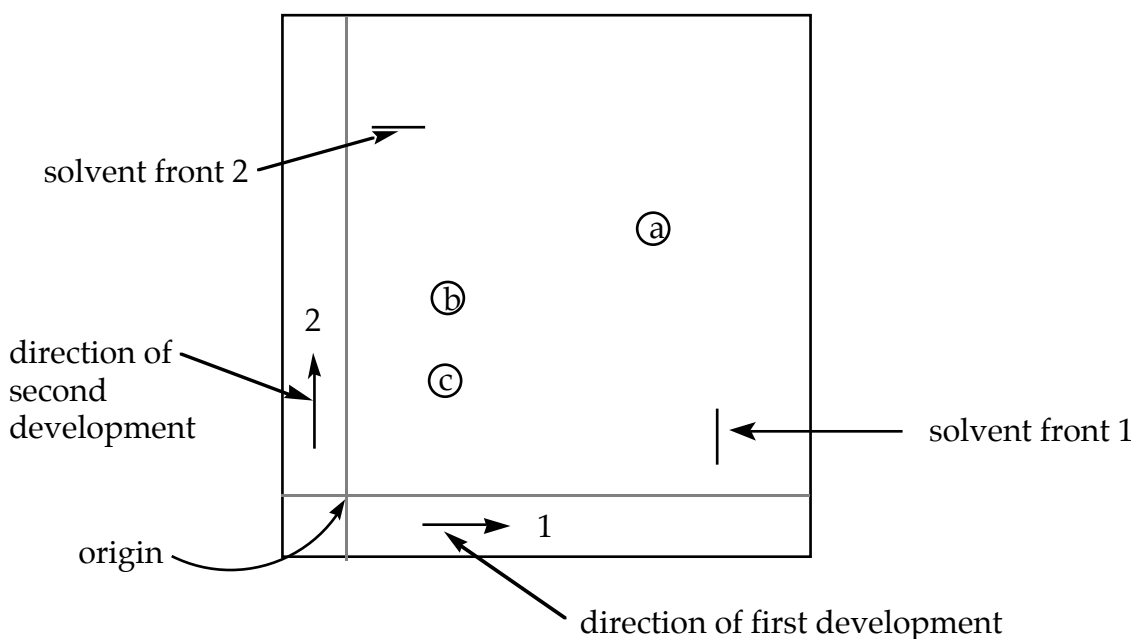


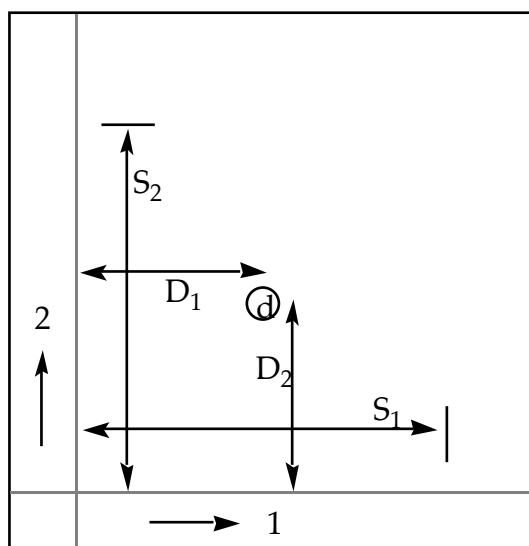
Figure 1

The mixture in Figure 1 has three components. The first development has separated compound **a** from compounds **b** and **c**, but not compounds **b** and **c** themselves. Thus, if this mixture was run using conventional TLC, only two spots would be seen. However, the second development has separated compound **b** from compound **c**, allowing all components of the mixture to be seen.

Because 2-D TLC plates have been developed twice, each spot will have **two**  $R_f$  values (see Figure 2). Note that the distance that the spot travels is measured from the **centre** of the spot. Thus, for compound **d** in Figure 2:

$$R_f(1) = D_1 / S_1$$

$$R_f(2) = D_2 / S_2$$



**Figure 2**

This experiment involves the analysis of a soil sample extract for polyaromatic hydrocarbons (PAH's) using 2-D TLC. PAH's are also commonly detected using gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC).<sup>1</sup>

**PAH's are highly toxic carcinogens. Nitrile (heavy green) gloves must be worn at all times and all experimental work involving PAH's must be carried out in the fumehoods.**

The TLC plates you will be using are both **expensive** and in **fairly short supply**. **If you have any doubts about running a TLC, consult a demonstrator.**

A Soxhlet apparatus will be used to extract the PAH's from the soil sample. Set up the Soxhlet extraction of your soil sample first, and while that is going, run the 2-D TLC of your standard. There are two different standard solutions; the one you will need will depend on your unknown. Unknowns 1.1 - 1.6 require Standard 1, while Unknowns 2.1 - 2.3 require Standard 2.

The standard solutions contain the following components (fluorescence colours under UV light in parentheses). **Fluorescence** occurs when a molecule excited by incident light emits radiation in order to return to the ground state. The colour of this emitted radiation (light) depends on the structure of the molecule. The fact that different molecules will often have different fluorescence colours is useful in telling them apart.

Standard 1 fluoranthene (light blue / blue-green)  
 3,4-benzofluoranthene (blue)  
 benzo(e)pyrene (purple)  
 fluorenone (yellow)

Standard 2 anthracene (purple)  
 3,4-benzofluoranthene (blue)  
 benzo(e)pyrene (purple)  
 fluorenone (yellow)

## Running a 2-dimensional TLC:

PAH's are light sensitive, so the development chambers must be protected from light. This is easily done by wrapping each chamber (and cover) in aluminium foil.

The two solvent systems you will be using are:

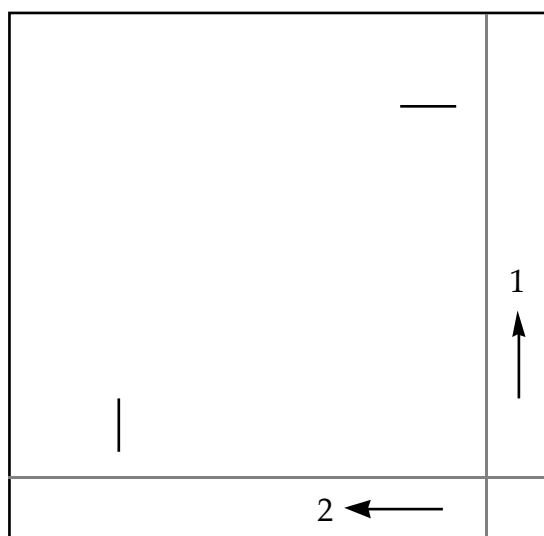
For the **first** development: hexane/chloroform (98:2)

For the **second** development: methanol/ether/distilled water (4:4:1)

Prepare 100 ml of the first solvent system (98 ml of hexane and 2 ml of chloroform) and 90 ml of the second solvent system (40 ml of methanol, 40 ml of ether and 10 ml of distilled water). Ensure each solvent system is thoroughly mixed.

Pour each development solvent to a depth of 1 cm (mark the outside of the chamber so you have some idea) into separate developing chambers, place the covers over the chambers and allow the solvent to equilibrate with its vapour for 15-20 minutes.

Using the straight edges of the TLC plate as a guide, *lightly* rule a pencil (do **not** use a pen!) line 1.5 cm from each of two edges. Place a light pencil mark 5 cm from each pencil line to give yourself an idea of how far the solvent needs to travel for each development. Mark (in pencil - lightly) the direction of travel for each development. Your TLC plate should now look something like this (Figure 3):



**Figure 3** (not to scale)

Spot the solution to be analysed at the intersection of the two pencil lines. A *single brief touch* should be all that is required.

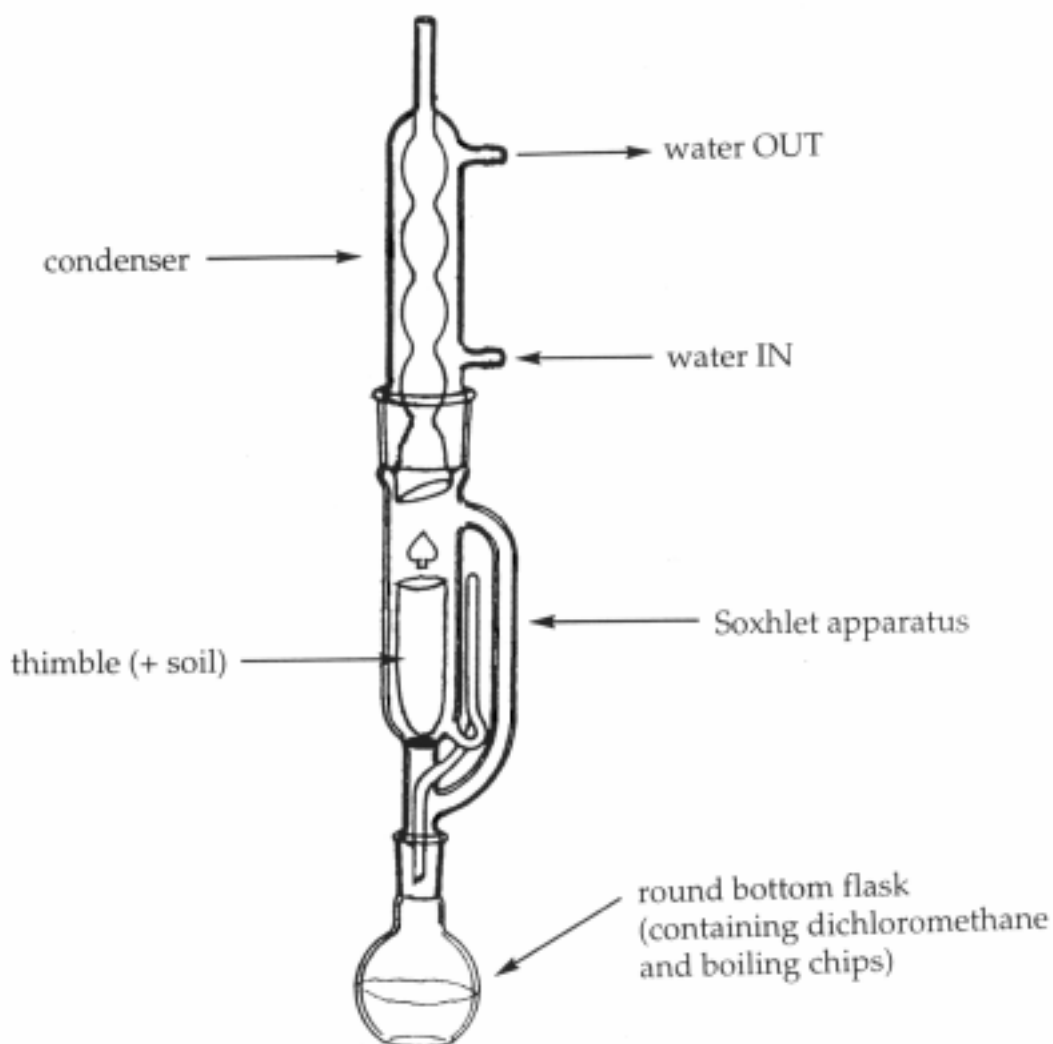
Place the TLC plate in the solvent system for the first development and allow the solvent to move up to a distance of approximately 5 cm from the origin (about 15 minutes - keep a close watch!). Mark the solvent front (if it differs from your earlier pencil mark). Allow the plate to dry thoroughly (about 2 min) then turn it on its side and develop in the second solvent system. Again, allow the solvent to move up approximately 5 cm from the origin (about 30 minutes) and mark the solvent front.

Dry the plate thoroughly and visualise the spots using UV light. Circle and number the spots in pencil and note their fluorescence colours.

Calculate  $R_f$  values (remember, each spot will have two) for each spot.

**Procedure for Soxhlet extraction:**

Set up the glassware as shown in Figure 4.



**Figure 4**

Tip the soil sample (don't leave any behind) into the Soxhlet thimble and place the thimble into the Soxhlet apparatus.

Using 75 ml of dichloromethane in the round-bottom flask (don't forget boiling chips) extract the soil sample for 45 minutes - 1 hour (approximately 6-8 "fills" of the Soxhlet reservoir, depending on the level of heating). Consult a demonstrator if you are unsure. You may have to add more boiling chips after each emptying of the Soxhlet reservoir. Consult a demonstrator about this.

Concentrate the extract (rotary evaporator or steam bath) to approximately half of its original volume and analyse by 2-D TLC.

Identify the two PAH's present in your soil sample (**record its number**) by comparing the 2-D TLC's of the extract and the standard.

Include diagrams of **both** your TLC plates in your report.

### **Waste Disposal:**

Ensure that **all** solvents and solvent mixtures are disposed of in the **correct** waste solvent container. If you are unsure, ask!

### **Questions:**

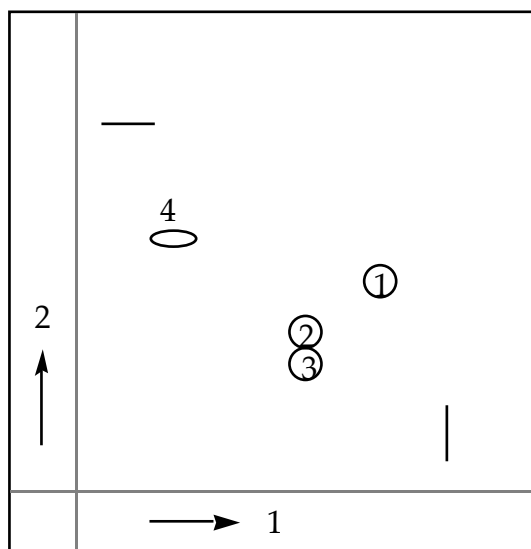
1. What are the structures of the PAH's present in your standard and soil sample?
2. Calculate  $R_f$  values for each component in your standard and soil sample extract.

### **Reference:**

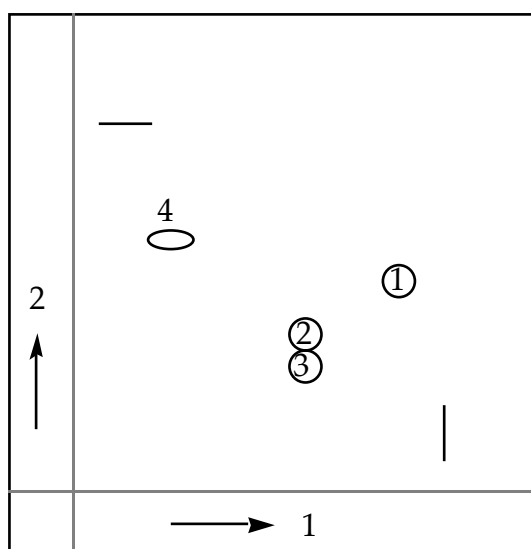
1. For PAH analysis by gas-liquid chromatography, see:  
<http://www.varian.com/inst/csb/gcnotes/gc16.html>  
For PAH analysis by high-performance liquid chromatography, see:  
<http://www.varian.com/inst/csb/hplcnote/lc08.html>

## Experiment 8 - Demonstrator notes

Standard 1 - fluoranthene (1, light blue / blue-green)  
3,4-benzofluoranthene (2, blue)  
benzo(e)pyrene (3, purple)  
fluorenone (4, yellow)



Standard 2 - anthracene (1, purple)  
3,4-benzofluoranthene (2, blue)  
benzo(e)pyrene (3, purple)  
fluorenone (4, yellow)



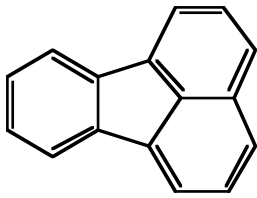
(fluorescence colours in parentheses)

- the TLC's are often streaky. Annoying, but can't be helped and doesn't interfere with identification of the compounds.

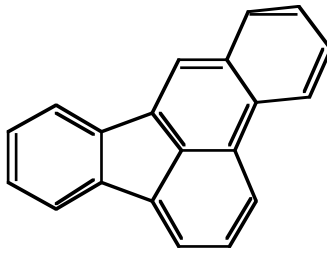
- sometimes the  $R_f$  values of the same components won't match for the standard and the unknown - possibly due to solvent evaporation in the time elapsed between running the standard and the unknown.  $R_f$ 's should be near enough to get a good idea and the colour of the spot will be the deciding factor.
- keep an eye on the students when they run their TLC's. Make sure they understand in which direction the 2nd development will have to be run. Also make sure that they use the correct solvent system for the first development (98:2 hexane/ $\text{CHCl}_3$ ).
- make sure the students mix the solvent systems **thoroughly** before pouring them into the developing chambers. They're told to do this in the manual, but some of them seem to think a quick shake in the measuring cylinder is enough! Get them to pour the solvent mixtures into the conical flasks provided and ensure they're well mixed. If the solvents aren't mixed properly, the PAH's in the standard and unknown won't separate well.
- don't let the students check on their TLC's every 5 minutes - there's no need (especially for the 2nd development which takes half an hour) and it just causes evaporation of solvent from the chamber.

#### Unknowns:

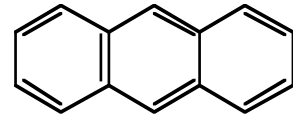
- 1.1 fluoranthene and 3,4-benzofluoranthene
- 1.2 fluoranthene and benzo(e)pyrene
- 1.3 fluoranthene and fluorenone
- 1.4 3,4-benzofluoranthene and benzo(e)pyrene
- 1.5 3,4-benzofluoranthene and fluorenone
- 1.6 benzo(e)pyrene and fluorenone
- 2.1 anthracene and 3,4-benzofluoranthene
- 2.2 anthracene and benzo(e) pyrene
- 2.3 anthracene and fluorenone



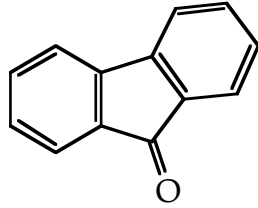
fluoranthene



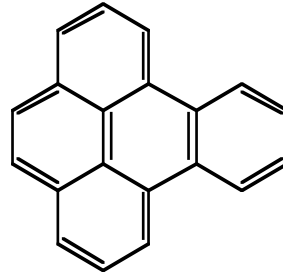
3,4-benzofluoranthene



anthracene



fluorenone



benzo(e)pyrene