Stir Bar Sorptive Extraction Analysis of PAHs in Aqueous Solution: Using a polymer based technique to pre-concentrate polyaromatic hydrocarbons for analysis by GC-MS.

Background:
Polyaromatic hydrocarbons (PAHs) are stable compounds that are widely distributed in environment. Several examples are shown below in Figure 1. They are formed from a variety of natural and anthropogenic sources and generally associated with incomplete combustion of organic materials. Because many PAHs are toxic and some are known to be carcinogenic, there is a need for convenient methods for quantitative analysis. Many of these quantitative methods involve chromatographic separation coupled to mass spectrometry. However, getting the analytes out of an aqueous sample and into a solvent which is compatible with various types of chromatography can be time consuming, laborious and require the use significant amounts of clean solvents.

![Figure 1: Structures of Selected Polyaromatic Hydrocarbons (PAHs)](image)

Although there are several well established analytical methods for PAHs, a number of solid phase and polymer based methods have recently emerged which provide convenient alternatives. Solid phase extraction (SPE) techniques use an octadecyl (C18) coated solid phase usually packed into small columns. The aqueous sample is passed through the column and the organic analytes are retained through adsorption. After drying, an organic solvent is passed through the column and the analytes are eluded. This process is sometimes referred to solvent exchange as the analytes originally in water are transferred into an organic solvent and ready for quantitation.

In solid phase micro-extraction (SPME) uses a polydimethylsiloxane (PDMS) polymer coated fibre which is inserted into the sample. Since the polymer coating is a relatively hydrophobic, analytes with
large aqueous activity coefficients effectively partition into the PDMS. The fibre which is usually mounted on the end of a syringe needle, is then directly placed into a heated gas chromatograph (GC) injection port whereupon the analytes are thermally desorbed onto a capillary column.

A simple alternative to the above sample preparation techniques involve a PDMS coated magnetic stir bar such as those supplied by Gerstel™. In this approach, known as stir bar sorptive extraction (SBSE), the PDMS coated stir bar is rapidly stirred in the sample until analytes are effectively extracted into the polymer coating.6-10 The stir bars can then be removed and thermally desorbed7,8 in the gas chromatograph injection port or back-extracted with an organic solvent9,10, which is then loaded onto an analytical column via syringe injection.

It can be shown6 that the recovery of the analyte can be estimated by the following equation;

\[
\frac{m_s}{m_o} = \frac{K_{ow}/\beta}{1 + (K_{ow}/\beta)}
\]

where \(m_s\) is the mass of analyte in the PDMS phase, \(m_o\) is the total amount of analyte in the original water sample, \(K_{ow}\) is the octanol-water partition coefficient, \(\beta\) is the phase volume ratio (\(\beta = V_w/V_s\); \(V_w\) and \(V_s\) are the volumes of the water sample and the PDMS phase, respectively).

The Gerstel Twister PDMS Coated (Type 1) stir bars that we are using in this lab have a cylindrical coating of 0.5 mm thickness and 10 mm length, corresponding to a volume of PDMS of 24 uL.

Mass spectrometric techniques provide both sensitive and selective quantitation with excellent linearity. Briefly, an analyte in the gas phase is ionized and these ions are transferred to a mass selective detector. Mass spectrometric signals can be recorded continuously over time at a number of different mass/charge channels, making it well suited as a chromatographic detection system. A series of selected ion monitoring (SIM) chromatograms are generated, each chosen to be selective for a particular analyte (see Table 1 for recommended SIM masses for selected PAHs).

Table 1: Physical Properties1 and Mass Spectrometry Selected Ion Monitoring2 (SIM) of Several PAHs

<table>
<thead>
<tr>
<th>PAH</th>
<th>Formula</th>
<th>MW (g/mol)</th>
<th>BP (°C)</th>
<th>Log K_{ow} (a)</th>
<th>SIM ion (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>C_{10}H_{8}</td>
<td>128</td>
<td>218</td>
<td>3.35</td>
<td>128</td>
</tr>
<tr>
<td>Naphthalene-d8</td>
<td>C_{10}D_{8}</td>
<td>136</td>
<td></td>
<td></td>
<td>136</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>C_{12}H_{10}</td>
<td>154</td>
<td>295</td>
<td>4.09</td>
<td>154</td>
</tr>
<tr>
<td>Fluorene</td>
<td>C_{13}H_{10}</td>
<td>166</td>
<td>342</td>
<td>4.18</td>
<td>166</td>
</tr>
<tr>
<td>Anthracene</td>
<td>C_{14}H_{10}</td>
<td>178</td>
<td></td>
<td>4.50</td>
<td>178</td>
</tr>
<tr>
<td>Pyrene</td>
<td>C_{16}H_{10}</td>
<td>202</td>
<td>404</td>
<td>5.00</td>
<td>202</td>
</tr>
</tbody>
</table>

\(a\) Evaluated \(K_{ow}\) values obtained from Sangster Research Laboratories, http://logkow.cisti.nrc.ca/logkow/index.jsp
**EXPERIMENTAL**

**Objectives:**
To use stir bar sorptive extraction and capillary gas chromatography mass spectrometry to analyze water samples for polyaromatic hydrocarbon contamination.

In this lab you will analyze:
- contaminated leachate sample
- blank deionized water sample
- standard reference sample containing selected PAHs (contains 5% methanol cosolvent)

**Procedure:**
Sample Blank:
- Gravimetrically transfer 100 g of sample into a 125 mL wide mouth glass vial sealed with a teflon backed seal.
- Add an 5 mL of methanol.
- Add a Gerstel™ PDMS coated stir bar and stir at high speed for 60 mins.
- Carefully remove stir bar with clean tweezers, dry with a lint free tissue and place in a 2 mL amber glass vial containing 500 μL of dichloromethane.
- Cap and place vial in sonicator for 15 mins.
- Remove stir bar and crimp a new cap on vial.
- Add 100 μL of an internal standard spike containing a naphthalene-d8.
- Inject 1 μL air segmented sample into GC-MS (see instructions below).

Contaminated leachate sample:
- Gravimetrically transfer 100 g of sample into a 125 mL wide mouth glass vial sealed with a teflon backed seal.
- Add an 5 mL of methanol
- Add a Gerstel™ PDMS coated stir bar and stir at high speed for 60 mins.
- Carefully remove stir bar with clean tweezers, dry with a lint free tissue and place in a 2 mL amber glass vial containing 500 μL of dichloromethane.
- Cap and place vial in sonicator for 15 mins.
- Remove stir bar and crimp a new cap on vial.
- Add 100 μL of an internal standard spike containing a naphthalene-d8.
- Inject 1 μL air segmented into GC-MS (see instructions below).

Standard reference sample:
- Volumetrically transfer 100. mL of sample into a 125 mL wide mouth glass vial sealed with a Teflon backed seal.
- Add a spike containing deuterated PAHs (see instructions in lab for details).
- Add a Gerstel™ PDMS coated stir bar and stir at high speed for 60 mins.
- Carefully remove stir bar with clean tweezers, dry with a lint free tissue and place in a 2 mL amber glass vial containing 500 μL of dichloromethane.
- Cap and place vial in sonicator for 15 mins.
- Remove stir bar and crimp a new cap on vial.
- Add 100. μL of an internal standard spike containing a naphthalene-d8.
- Inject 1 μL air segmented into GC-MS (see instructions below).
**GC-MS Calibration:**
Using the supplied combined PAH standard stock solution, make up a combined PAH calibration solution (in methanol) containing selected PAHs in 100 – 500 µg/L concentration range. Transfer 1.00 mL of this calibration standard to a 2 mL amber glass GC vial and add 100. µL of internal standard. Cap with a Teflon lined septa cap. (You may use gravimetric dilutions to make up these standards, if you like. Remember 1 µg/L = 1 ppb in water where the density is 1.00 kg/L. In other solvents, you must keep track of concentration units carefully. To avoid possible confusion, I would recommend that you report all concentrations in non-aqueous solvents as mass/volume, such as µg/L rather than ppm or ppb).

Using the supplied GC-MS parameters, inject 1 µL of your of your combined standard as an air segmented plug. Record the total ion count (TIC) and a series of selected ion monitoring (SIM) chromatograms. Identify and record the retention times of each PAH using the SIM chromatograms. (See your instructor for injection and instrument operation).

**GC parameters:** Column 30m, RTX-5 MS, 0.25 mm ID, 0.3um film thickness
- injection volume 1 µL (air segmented plug)
- injector temperature 270°C
- oven temp program 100°C (2 mins); ramped 25°C/min to 240°C; ramped 10°C/min to 280°C (hold 2 mins)
- transfer line 200°C
- He flow rate 0.5 mL/min
- split mode 1:10

**MS parameters:** Datafile; C331_01.ms
- full scan mode
- mass range 50 - 350 m/z
- scan time (3 µScans) 0.73 sec
- MS temp 200°C

**Data Analysis:**
- Using the peak areas from the SIM chromatograms for the combined standard, calculate a relative response factor (RRF) for each PAH relative to the deuterated naphthalene internal standard.
- Determine the concentration of selected PAHs in the aqueous samples.
- Calculate the % recovery of PAHs using the reported SRM concentration levels.

**Questions:**
1. How do the values of K_{ow} compare to K_{pdlms-w}?  
2. How does the K_{ow} of various analytes relate to the recoveries using SBSE?  
3. Comment on how the sample and extraction solvent temperature will influence SBSE efficiency?  
4. How would adding salt to the original water sample affect the aqueous activity coefficients and the analyte recovery.
5. Suggest any other experimental modifications that would improve the recovery and/or the sensitivity of SBSE.

References:


4.) M. S. Garcia-Falcon., M. Perez-Lamela, J. Simal-Gandara, Comparison of Strategies for Extraction of High Molecular Weight Polycyclic Aromatic Hydrocarbons from Drinking Waters, *J. Agric. Food Chem.*; (Article); 2004; 52(23); 6897-6903. SPE


