The Octanol-Water Partition Constant: Using HPLC Retention Times to Estimate an Important Physicochemical Parameter

Background:
The fate of organic molecules (whether natural or anthropogenic) is governed, in part, by their distribution in the environment. The distribution of molecules between various environmental phases (e.g., air, water, soils and biota) is a physical process, which occurs without a chemical change to the molecule itself. How a molecule distributes in the environment is determined by it’s intrinsic physicochemical properties which are largely a result of the nature and strength of the inter-molecular forces present. For example, the distribution of organic compounds between water and natural solids (e.g., soils, sediments and suspended particles or biological organisms) can be viewed as an equilibrium partitioning process between the aqueous phase and the bulk organic matter present in natural solids or biota. It has been observed, that ‘water-immiscible’ organic solvents like n-octanol can be used as a surrogate for biological systems in estimating the distribution of organic molecules between water and organisms (1). Although the extent of uptake from water into n-octanol is not identical as that in organisms, in most cases it appears to be directly proportional; that is, within a series of structurally related compounds, greater partitioning into n-octanol from water corresponds to higher accumulation into the organism (i.e., a higher bio-concentration factor). More recently, environmental chemists have found similar correlations with soil humus and other naturally occurring organic phases. These correlations exist because the same molecular factors controlling the distribution of compounds between organic solvents and water also determine environmental partitioning from water into natural organic phases.

The partitioning process at equilibrium is described by a dimensionless equilibrium constant:

\[
K_{ow} = \frac{C_o}{C_w}
\]

where \(K_{ow}\) is the octanol-water partition constant, \(C_o\) is the concentration of the organic compound in \(n\)-octanol and \(C_w\) is the concentration of the organic compound in water. The figure below shows ranges of \(K_{ow}\) for some common xenobiotic compound classes (2).
Traditionally, $K_{ow}$ measurements have been carried using the ‘shake flask’ method whereby an organic solute is left to equilibrate between two immiscible solvents $n$-octanol and water in a closed container. The concentration of the compound is then analyzed by conventional techniques in each phase (3). This method can be very time consuming and is best suited for compounds with low to medium $K_{ow}$ values. For hydrophobic compounds, accurate $K_{ow}$ measurements are limited by self-aggregation phenomena and poor analytical detection in the aqueous phase. This problem has been partially addressed with the use of ‘generator columns’ coupled with solid sorbent cartridges (4). However, reliable $K_{ow}$ data for highly hydrophobic compounds remains a significant challenge and it is not uncommon to find values in the literature that differ by as much as 2-3 orders of magnitude (5).

$K_{ow}$ values have been estimated using linear free energy relationships (LFERs) for a series of structurally related compounds and correlations with aqueous solubility ($C_{w}^{Sat}$). Another approach to estimate the $K_{ow}$ of a given compound is based on the retention behaviour of the compound in a chromatographic system, particularly HPLC (high performance liquid chromatography) (6-7). Here the organic solute is transported in a polar mobile phase through a porous stationary phase which exhibits hydrophobic properties as typically used in reversed-phase chromatography. The solute partitions between the mobile phase ($methanol$-$water$) and the stationary phase (typically an octyldecylsilane: 18 carbon $n$-alkane bonded to a silica support). The $C_{18}$ hydrocarbon provides a hydrophobic micro-environment and compounds moving through this system, partition between the stationary (non-polar) and mobile (polar) phases according to their physicochemical properties. The retention time of the solute (the time the solute is retained by the stationary phase on the column) is determined by the partition constant of the solute between the mobile and stationary phases.

Since the nature and strength of the solute-solute and solute-solvent interactions that control the $n$-octanol and water solubilities also affect the partitioning between the polar mobile and $C_{18}$ stationary phases in the HPLC, there is a good correlation between $K_{ow}$ and retention time. The dominant term in the thermodynamics (both enthalpy and entropy) of dissolution for neutral organic compounds is molecular size as measured by the total surface area (see further, Schwarzenbach et. al., figs. 5.3, 5.4 & 5.5). However, the interaction of solutes with the $C_{18}$ phase occurs by an adsorption mechanism and the retention on an HPLC column is also determined by the effectiveness of the contact between the solute and the $C_{18}$ phase. As a consequence, good correlations between retention time and $K_{ow}$ are obtained for classes of compounds with similar molecular shapes.
EXPERIMENTAL

Objectives:
- Obtain HPLC retention times (capacity factor) for a series of hydrophobic organic compounds for which the $K_{ow}$ values are known.
- Correlate the retention time data to $K_{ow}$ values to generate a calibration curve.
- Use the correlation generated above to predict the $K_{ow}$ values for compounds with ‘unknown’ values.

Procedures:

Solution Preparations

Stock solutions (~500 ppm) of each of the following compounds will be supplied in HPLC grade methanol: anthracene, benzene, biphenyl, bisphenol-A, bromobenzene, 1-chloronaphthalene, p-dichlorobenzene, dieldrin, dibutyl phthalate, fluorene, naphthalene, pyrene and toluene.

Prepare diluted solutions (10-50 ppm) of each individual compound by pipeting 1000 uL of the stock solution into a 7 mL amber glass vial and diluting to ~5 mL with HPLC grade methanol.

Prepare two combined solutions suites (10-50 ppm of each compound) by pipetting 700 uL of each compound into a 7 mL amber glass vial (note: some compounds are included in both combined solutions).

Combined Suite #1:
anthracene, biphenyl, bisphenol-A, bromobenzene, 1-chloronaphthalene, fluorene, naphthalene and pyrene.

Combined Suite #2:
anthracene, benzene, biphenyl, dibutylphthalate, p-dichlorobenzene, dieldrin, pyrene and toluene.

In order to determine the identity of each peak in the HPLC runs of the combined solutions, each compound should be run individually. Each group will run four individual compounds and both combined solution suites. Working as a class, we will combine individual retention compound retention time data to unambiguously assign each peak in the combined solution suite runs (note: to avoid ambiguity in the peak assignments, two combined solutions are prepared and run separately).
**HPLC Injections**

Prior to injecting samples into the HPLC it is important to remove particulates. Pass ~1 mL of each solution to be injected through a 0.2 µm membrane syringe filter and into the 20 µL HPLC injection loop (see Figure below).

Both HPLCs are set up with a hydrophobic C$_{18}$ column (Restek Pinnacle DB C18, 3um, 100x4.6 mm), an isocratic 70% (vol/vol) methanol-water mobile phase, and the column flowrate set to mL/min. Detection is by UV absorbance: the *D-Star* isocratic HPLC has a fixed wavelength detector set to 254 nm, and the *SSI* binary HPLC is attached to a tunable wavelength detector set to 220 nm. The extinction coefficient of organic molecules varies with wavelength. Most compounds absorb sufficiently at both 220 and 254 nm to be detected, dieldrin absorbs weakly at 254 nm and may only be detectable on the *SSI* system set at 220 nm.

To account for operational differences in HPLC, we will actually use the capacity factor instead of the retention time. The capacity factor ($k'$) is the retention time of a compound relative to a non-retained chemical species:

$$k' = \frac{t - t_0}{t_0}$$

where $k'$ is the capacity factor, $t$ the retention time and $t_0$ the retention time of the non-retained species. In this case we will use the time required for solvent front ‘peak’ as $t_0$. 
Calculations:
The following table gives literature log $K_{ow}$ values. Using this set of compounds to as a ‘training set’, determine the equation for the linear regression of log $K_{ow}$ and log $k'$. Use the retention time of ‘test’ compounds bromobenzene, bisphenol-A, dibutyl phthalate and dieldrin to predict their log $K_{ow}$ values.

Evaluated Literature $K_{ow}$’s (Training Set)

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS #</th>
<th>log $K_{ow}$ (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>71-43-2</td>
<td>2.13</td>
</tr>
<tr>
<td>Toluene</td>
<td>108-88-3</td>
<td>2.73</td>
</tr>
<tr>
<td>p-Dichlorobenzene</td>
<td>106-46-7</td>
<td>3.45</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>91-20-3</td>
<td>3.35</td>
</tr>
<tr>
<td>Fluorene</td>
<td>86-73-7</td>
<td>4.18</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>92-52-4</td>
<td>3.98</td>
</tr>
<tr>
<td>Anthracene</td>
<td>120-12-7</td>
<td>4.50</td>
</tr>
<tr>
<td>Pyrene</td>
<td>129-00-0</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Unknown $K_{ow}$’s (Test Compounds)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bromobenzene</th>
<th>Bisphenol-A</th>
<th>Dibutylphthalate</th>
<th>Dieldrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS #</td>
<td>108-86-1</td>
<td>80-05-7</td>
<td>84-74-2</td>
<td>60-57-1</td>
</tr>
<tr>
<td>log $K_{ow}$</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

Question:
Compare your predicted value of log $K_{ow}$ for bromobenzene, bisphenol-A, dibutyl phthalate and dieldrin with the literature and comment on this strengths and limitations of this approach for these compounds.

References:
(8) LOGKOW: A databank of evaluated octanol-water partition coefficients (Log P), Sangster Research Laboratories; [http://logkow.cisti.nrc.ca/logkow/index.jsp](http://logkow.cisti.nrc.ca/logkow/index.jsp)