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

Background:

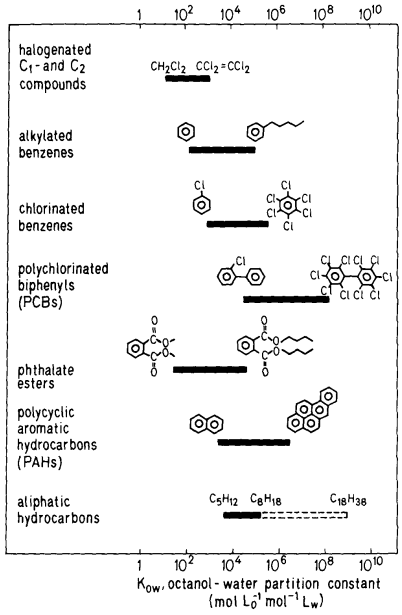
The fate of organic molecules (whether natural or anthropogenic) is governed, in part, by their distribution in the environment. The distribution of molecules between various environmental phases (e.g., air, water, soils and biota) is a physical process, which occurs without a chemical change to the 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:

$$X_{(aq)} ==== X_{(octanol)}$$

$$K_{ow} = \frac{C_o}{C_w}$$

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-chloronapthalene, p-dichlorobenzene, dieldrin, dibutyl phthalate, fluorene, naphthalene, pyrene and toluene.

Prepare diluted solutions (10-50 ppm) of each individual compound by pipeting 300 uL of the stock solution into a 7 mL amber glass vial and diluting to ~6 mL with HPLC grade methanol.

Prepare two combined solutions suites (10-50 ppm of each compound) by pipetting 300 uL of each compound into a 7 mL amber glass vial and diluting to ~6 mL with HPLC grade methanol (note: some compounds are included in both combined solutions).

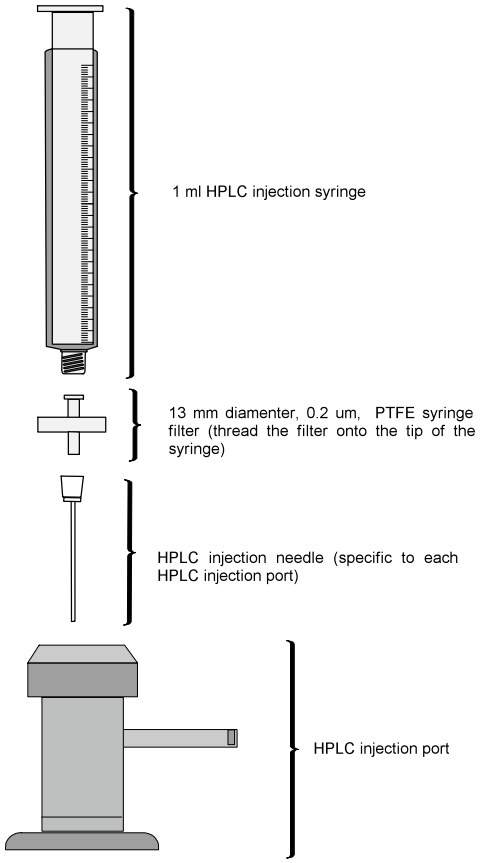
Combined Suite #1:
anthracene, biphenyl, bisphenol-A, dibutyl phthalate, dieldrin, fluorene, naphthalene and toluene.

Combined Suite #2:
anthracene, benzene, biphenyl, bromobenzene, 1-chloronapthalene, p-dichlorobenzene, fluorene and pyrene.

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).



Both HPLCs are set up with a hydrophobic C_{18} column (stationary phase) and an isocratic 70% (vol/vol) methanol-water mobile phase at a flowrate of 1 mL/min. Detection is by UV absorbance. Note specific operating parameters and instrument specifics in the lab and use the same HPLC instrument for all runs in this experiment.

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 (i.e., solvent front);

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

where k' is the capacity factor, t the retention time and t_o the retention time of the non-retained species. In this case we will use the time required for solvent front 'peak' as t_o.

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)

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

Unknown K_{ow}'s (Test Compounds)

Compound	Bromobenzene	Bisphenol-A	Dibutyl phthalate	Dieldrin
CAS #	108-86-1	80-05-7	84-74-2	60-57-1
log K_{ow}	?	?	?	?

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:

(1) Chiou, C.T.; Freed, V.H.; Schmeddling, D.W.; Kohnert, R.L. Partition coefficient and bioaccumulation of selected organic chemicals, *Environ. Sci. Technol.,* **1977**, *11,* 475-478.
(2) **Environmental Organic Chemistry**, R.P. Schwarzenbach; P.M. Gschwend; D.M. Imboden, Wiley-Interscience Publishers, 1993, chapter 7.
(3) Leo, A.; Hansch, C.; Elkins, D. Partition coefficients and uses, *Chem. Rev.*, **1971**, *71*, 525-553.
(4) Tewari, Y.B.; Miller, M.M.; Wasik, S.P.; Martine, D.E. Aqueous solubility and octanol-water partition coefficeinets of organic compounds at 25°C, *J. Chem Eng. Data*, **1982**, *27*, 451-454.
(5) Renner, R. The K_{ow} Controversy, *Environ. Sci. Technol.,* **2002,** *36,* 411A-413A.
(6) Veith, G.D.; Austin, N.M.; Morris, R.T. A rapid method for estimating log P for organic chemicals, *Water Res.*, **1979**, *13*, 43-47.
(7) R. A. Rapaport, R.A.; Eisenrelch, S.J. Chromatographic determination of octanol-water partition coefficients (Kow's) for 58 PCB polychlorinated biphenyl congeners, *Environ. Sci. Technol.*, **1984,** *18,* 163- 170.
(8) LOGKOW: A databank of evaluated octanol-water partition coefficients (Lop P), Sangster Research Laboratories; http://logkow.cisti.nrc.ca/logkow/index.jsp