# ♣EPA  ENVIRONMENTAL RESEARCH BRIEF

## Measuring Octanol/Water Partition Coefficients by the "Slow-Stirring" Method

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## Abstract

A method for measuring the octanol/water partition coefficients ($K_{ow}$s) is described. The "slow-stirring" method minimizes the formation of emulsions and enables the laboratory measurement of chemicals with a log $K_{ow}$ greater than 8. The techniques and equipment are described.

## Introduction

The octanol/water partition coefficient ($K_{ow}$) is the physicochemical parameter most commonly measured in the laboratory and used to characterize quantitatively the hydrophobic nature of organic compounds. The currently accepted EPA method for measuring $K_{ow}$ is the flask-shaking procedure described in the Federal Register [40 CFR Part 796, 50 (188) Friday, September 27, 1985, pp 39252-39255]. The EPA method is applicable for the range of $K_{ow}$s from $10^1$ to $10^6$. Significant variability appears, however, among measurements for compounds having $K_{ow}$s greater than $10^6$. This variability has been attributed to emulsion formation during the shaking step that can cause wide disparities in the concentrations of the solute in the water phases. The slow-stirring method (1-3) avoids the formation of emulsions, and the upper range of $K_{ow}$ measurement is limited only by the ability to determine the water phase concentration accurately. In this document, the slow-stirring methodology is described in detail and the use of Empore™ disks to extract hydrophobic chemicals from water volumes up to 8 liters is illustrated as a replacement for extraction of the test chemicals with solvents.

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## Definitions and Units

The octanol/water partition coefficient ($K_{ow}$) is defined as the ratio of the equilibrium concentration of a chemical in the octanol phase to that in the aqueous phase. The concentrations are expressed in terms of the mass or moles of chemical per unit volume of liquid.

$$K_{ow} = C_{octanol}/C_{water} \qquad (1)$$

Log $K_{ow}$ is the value most often reported in the literature. Log $K_{ow}$ is used in the calculation of bioaccumulation factors, and for estimating the organic carbon-normalized sediment/water partition coefficient ($K_{oc}$) and for calculating water solubility and other properties using property reactivity correlations.

## Experimental

### Equipment

An Erlenmeyer flask or cylindrical jar of appropriate size to permit withdrawal of sufficient sample for precise measurement is modified and used for the equilibration of the test solute between octanol and water. One-liter Erlenmeyer flasks are used if the expected log $K_{ow}$ is less than 3. Larger vessels (6- or 9-liters) are needed if the log $K_{ow}$ is greater than 3. Figure 1 shows a cylindrical jar that has been modified for use with this method by installing a 2-mm straight bore stopcock with a Teflon plug through the container side wall at a point near the bottom. The stopcock is inserted near the bottom so that the maximum volume of water layer can be withdrawn. The 2-mm glass tube on the side of the Teflon plug away from the flask is turned down to form a tap for withdrawing the aqueous layer. A magnetic stirrer with stir bar is needed for each flask. The stirrers should be of appropriate size with the speed adjusted
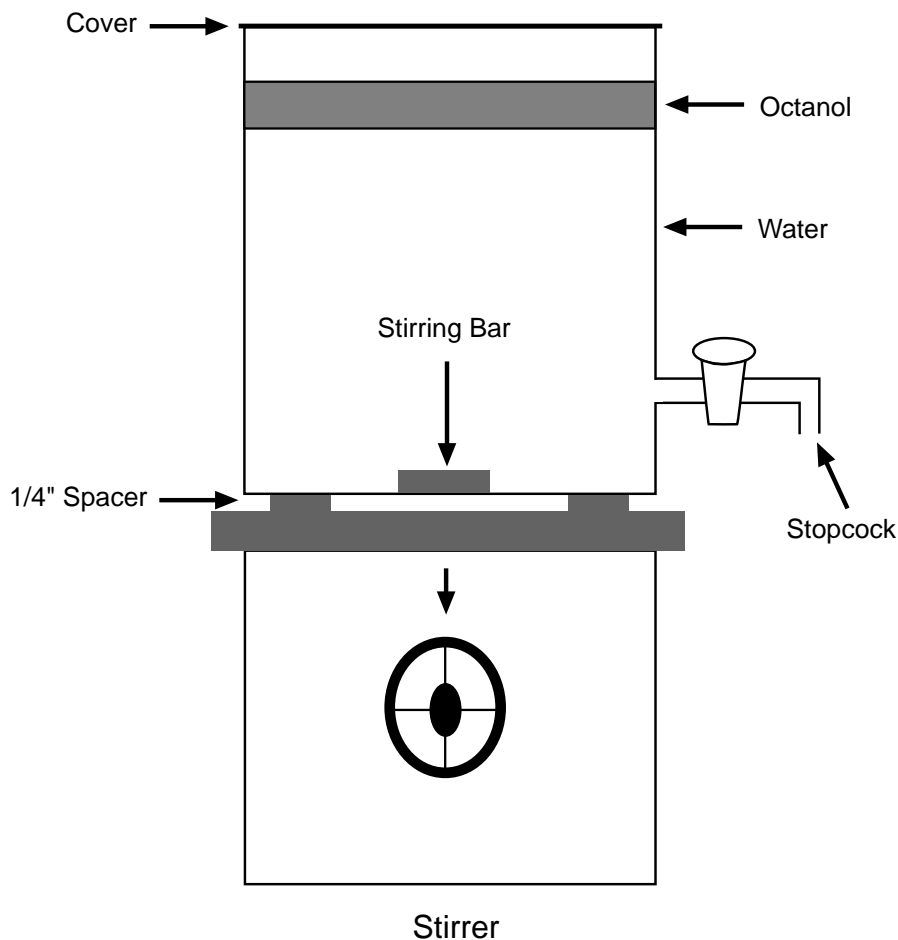
Cover → ▭

Octanol ←

Water ←

Stirring Bar ↓

1/4" Spacer →

Stopcock ↗

Stirrer

*Figure 1.    Equilibration vessel equipped with Teflon stopcock.*

such that a ≤ 2-cm deep vortex is generated at the octanol/water interface.

### Temperature Control
The temperature of the measurement vessel and contained liquids should be maintained at 25(±1)°C. The vessel can be either placed in a temperature-controlled room or the vessel can be fabricated such that water of a constant 25°C flows continuously across the exterior wall of the vessel.

### Chemicals
It is important that the octanol used be of 99+% purity. The water used should be American Society for Testing and Materials Type I, produced by laboratory water purification systems. Solvents used in the preparation of calibration solutions and for extraction of the test chemical from the water layer must be of the highest quality commonly used for trace analysis of environmental samples.

A check on the purity of the octanol and other solvents used and the identification and measurement of volatile impurities should be made by gas chromatography/mass spectrometry (GC/MS). The identities of nonvolatile compounds are confirmed by comparison of their ultraviolet, infrared or other appropriate spectra to library reference spectra. Nonvolatile test compounds are analyzed by high performance liquid chromatography/mass spectrometry (HPLC/MS) when available.

### Octanol/water volume ratio
At least 20 ml of octanol should be used for each liter of water (1:50).

### Chemical concentration in octanol
The solute should be <0.01 M in octanol and not exceed 50% of the solute solubility in either phase.

### Analysis
Analysis systems based on chromatography, such as gas chromatography (GC) and HPLC, are preferred for test solute measurement in the octanol and water phases. The choice of the detector is determined by the physical and chemical properties of the solute. Commonly used detectors are flame ionization, electron capture and nitrogen-phosphorus for GCs. Ultraviolet and fluorescence detectors are used for HPLCs. The concentration of a >106 $K_{ow}$ solute in the water layer extract will be in the low parts per billion. Therefore, analysis of these extracts will require efficient chromatographic columns

and sensitive detectors capable of a reproducible response to nanogram/microliter injections of the solute. GCs equipped with autosamplers, and LCs equipped with loop injectors yield the highest precision injections and should be used when available. Detector response of triplicate manual injections should not deviate by more than ±3% relative standard deviation. The minimum acceptable detection level of the chromatographic analysis is established by triplicate injections of serial dilutions of the stock solution of the solute. The minimum acceptable detection level is defined as the minimum concentration or mass flux of analyte (in a solvent) that gives a detector signal that can be discerned from the noise with reasonable certainty, generally recognized to be twice the peak-to-peak noise (4). As explained in more detail in the section on calculation of $K_{ow}$, it is not necessary to determine the absolute concentration of the solute in the two phases. Analysis of calibration standards is performed only to ensure that the amount of solute analyzed for determination of the $K_{ow}$ is within the linear range of the detector. The calibration standards are not used to determine the absolute test solute concentration in the octanol and water phases.

An error in the measured $K_{ow}$ can occur if a nonchromatographic method, such as radiolabeling or UV absorption spectroscopy, is used to determine response of the test solute in the two layers. These errors result from the presence of traces of more-water-soluble contaminants that are not analytically distinguished from the test solute. If nonchromatographic methods are used, the error introduced by the water-soluble contaminants can be minimized by subjecting the test solute-containing octanol phase to consecutive equilibrations with water. The equilibrations should be repeated with new water phases until the $K_{ow}$ is unchanged for two consecutive equilibrations.

## Calculation of $K_{ow}$

It is not necessary to determine the absolute concentration of the test solute in either phase. The $K_{ow}$ can be calculated directly by dividing the linear detector response to equal volumes of the octanol and water phases, respectively. In theory, the $K_{ow}$ of a test solute analyzed by GC can be calculated by dividing the solute detector response to a 1-μl injection of the octanol layer by the solute detector response to a 1-μl injection of the water layer. However, for solutes with log $K_{ow}$ greater than 1, the concentrations in the two phases differ by orders of magnitude and usually exceed the dynamic range of the detector. To minimize this difference in concentrations and to obtain samples from the two phases that will give approximately the same detector response when equal volumes are analyzed, an aliquot of the octanol layer is diluted and the test solute in the water layer is concentrated by an appropriate extraction technique. Extraction of the test solute with a solvent is used for water samples ≤1 L; solid sorbent extraction is used for water samples >1 L. The $K_{ow}$ for test solutes with $K_{ow}$ greater than 1 is then calculated by the following equation:

$$K_{ow} = \frac{(\text{Detector response})_{octanol} \times \text{Dilution Factor}}{(\text{Detector response})\text{water} \div \text{Concentration Factor}} \quad (2)$$

(If unequal volumes of the final octanol dilution or water concentration are analyzed, the unequal volumes must be taken into consideration when determining the detector response.)

## Procedure

At the beginning of the measurement procedure, the appropriate volume of Type I water is brought into the vessel together with a Teflon-coated magnetic stirring bar. One-liter water samples are stirred for 1 hour to equilibrate the temperature to 25°C; 5L and larger samples are stirred overnight. The surface of the stirrer warms during the equilibration time; to minimize heat transfer, three to four 1-inch-square by 1/4-inch-thick pieces of wood are placed between the flask and the surface of the stirrer. When the water has reached an equilibrium temperature of 25°C, the stirring is stopped and pure octanol equal in volume to 25% (10 ml for 1L and 25 ml for 6L flasks) of the final volume is carefully added without mixing the phases. The addition of octanol is accomplished by tilting the flask and gently pouring the octanol down the interior surface of the vessel so that the octanol flows slowly onto the surface of the water.

Stirring is resumed and the stirring rate is adjusted until a vortex of approximately 2 cm is formed at the octanol/water interface. Stirring is continued overnight to achieve mutual saturation of the phases. The stirring is again stopped and the remaining 75% volume of octanol, containing the test solute of interest, is carefully layered onto the surface of the flask contents as described previously. Stirring is then resumed and is stopped only for taking samples. The overnight prestirring of the pure phases without test solute allows sufficient time for mutual saturation of the two phases. If the two phases are first contacted at the start of the test solute equilibration, the octanol that initially migrates into the water phase can also transport solvated solute molecules, thereby elevating the test solute concentration in the water phase to levels higher than the final equilibrium solute concentration in water. De Bruijn and co-workers (1) recognized the problems of test solute equilibration with unsaturated octanol and water phases. Mutual presaturation of the octanol and water phases described as the preferred method above is based on the work of Marple (3).

If the expected log $K_{ow}$ is less than 3, samples are taken on successive days (after the first 24 hours) until the $K_{ow}$ becomes constant. The sampling regime is at 2 to 3 day intervals for compounds with log $K_{ow}$ greater than 3. Since the approximate concentration of the solute at time zero in the octanol layer is known, the estimated equilibrium water concentration can be calculated from the equation (2) used to calculate $K_{ow}$ if an estimate of the $K_{ow}$ of the test compound is also available. The volume of water that must be withdrawn from the flask and extracted is determined based upon the calculated equilibrium water concentration and the analysis method sensitivity. The volume of the water sample required for the analysis is calculated by substituting the estimated $K_{ow}$ and the starting $C_{octanol}$ into Equation 1 and solving for $C_{water}$. The water sample taken should be of sufficient volume that the final concentration of the solute in the extraction solvent will be sufficient for analysis by the detection systems used.

The water layer is sampled by stopping the stirring and withdrawing a sample of water from the Teflon tap after discarding the first 25 ml. The water is collected in a volumetric flask. A 1-ml sample of the octanol is obtained by placing the tip of a 1-ml pipette just below the surface of the octanol and applying gentle suction. The octanol is diluted with the final solvent used in the extraction of the test solute from the water layer. The octanol is diluted until a detector response approximately equal to the response of the water layer extract is obtained.

Nonpolar solutes are concentrated from the water layer by conventional methods such as solvent extraction using methylene chloride or use of Empore™ extraction disks. The extraction solvents are usually concentrated to a final volume of 1 ml.

The dilution and concentration factors are calculated and used in equation 2 to calculate the $K_{ow}$.

## References

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