

CS-1420
October, 1985

PARTITION COEFFICIENT (n-OCTANOL/WATER)
GENERATOR COLUMN METHOD

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I. NEED FOR THE TEST

The octanol/water partition coefficient (K_{OW}) is one of the most frequently measured and most widely used parameters in assessing the environmental fate of organic chemicals. Its importance is due primarily to the fact that K_{OW} is used to predict the general lipophilicity or hydrophobicity of a chemical. Of greater significance on the quantitative level, however, is the fact that K_{OW} has been found to correlate with the water solubility, the soil/sediment sorption coefficient and the bioconcentration factor.

Although all of the above mentioned properties are important in predicting the fate and residence time of a chemical in the environment, the bioconcentration factor (BCF) carries with it the greatest consequences. This is because the BCF is used to predict the potential for a chemical to bioconcentrate in living tissue. When applied to the aquatic environment, the BCF is indicative of the potential for a chemical to bioconcentrate in the tissue of fish and other lower aquatic organisms. In terms of the K_{OW} , chemicals with a value less than 10 will not bioconcentrate while those with a value greater than 10^4 will. Thus, although a chemical may be present in a stream or lake at subtoxic concentrations, if its K_{OW} is greater than 10^4 it could bioconcentrate and accumulate to levels that may be toxic to not only the organism itself, but also to the consumers of that organism, not the least important of which are Homo sapiens.

The direct determination of K_{ow} , using the conventional shake-flask method, CS-1400 (EPA, 1983), is subject to numerous experimental difficulties. These include the formation of colloidal dispersions (emulsions) during the shaking step, incomplete separation of the octanol and water phases, difficulty in analyzing very hydrophobic chemicals in the water phase, the adsorption of the solute onto the surfaces of transfer vessels, and the loss of volatile solute into the atmosphere. In addition, the method is time consuming.

II. SCIENTIFIC ASPECTS

A. Rationale for the Test Method

This test method uses a chromatographic technique for determining the octanol/water partition coefficient. The method, called the generator column method, was developed and validated by Wasik et al (1981) and Tewari et al (1982); using this method K_{ow} is calculated as the ratio of the molar concentration of a 1.0% (w/w) solution of the test substance in octanol to the molar concentration of the test substance in water, at equilibrium. The advantages of the generator column method include: (1) all the difficulties of the shake-flask method are avoided; (2) equilibrium is achieved rapidly; (3) easy to carry out and gives precise and accurate results ($\pm 3\%$), especially for very hydrophobic chemicals and (4) is applicable to a wide range

of chemicals with different functional groups and over a wide range of $\log K_{ow}$ (1 to >6).

Wasik et al (1981) and Tewari et al. (1982) have also provided a thermodynamic basis for their method which was validated on 62 organic test substances falling into seven general chemical classes. Their validation studies showed excellent agreement between the values of K_{ow} obtained using the generator column method and those obtained using the standard shake flask method. Their results established that the data obtained using the generator column are precise and accurate to $\pm 3\%$ and that their method is the best currently available method for determining K_{ow} .

B. Other Methods for Determining K_{ow}

1. The Conventional Shake Flask Method

The conventional method for determining the octanol/water coefficient involves shaking a solute with two immiscible solvents and then measuring the solute concentration in the two solvents after equilibration (Leo, et al. 1971). The distribution of the solute between the two solvents is a direct consequence of the thermodynamic requirements for equilibrium that apply only to dilute solutions. The resulting ratio of the two solute concentrations is the partition coefficient which is constant at a given temperature. Numerous researchers have used this method and published their results (Fujita et al. 1964;

Hansch and Anderson 1967; Leo et al. 1971; Chiou et al. 1977). Both EPA (1983) and OECD (1981) specify the use of this method in their protocols (see CG-1400). Although there is no ASTM Standard Test Method for K_{ow} , the shake-flask method is the "standard method" used in industry when K_{ow} is to be determined.

As noted earlier, the shake flask method is not without problems, the most important of which are the formation of emulsions, surface adsorption of the solute, and the time consuming nature of the test procedure. However, with suitable care and patience these problems are minimized and basic experimental data are obtained that can be correlated with other important environmental fate parameters.

2. Reverse-Phase High-Pressure Liquid Chromatography as a Method of Estimating K_{ow}

A rapid method based on reverse-phase high-pressure liquid chromatography has been developed by Veith (Veith et al. 1979) to estimate the octanol/water partition coefficient of organic chemicals. This method has been incorporated into test guideline CG-1410: Partition Coefficient (n-Octanol/Water) Estimation by Liquid Chromatography (EPA, 1983). A summary of the method follows. Using the solvent mixture water/methanol (15/85 v/v) as the elutant, the log of the retention time [$\log(t_R)$] of organic chemicals on a permanently bonded (C-18) reverse-phase high-

pressure liquid chromatographic system has been found to be linearly related to $\log K_{OW}$. This relationship has been expressed by the equation

$$\log K_{OW} = A \log (t_R) - B, \quad (1)$$

where A and B are constants determined from the experimental data for some organic chemicals. Using a mixture of the chemicals benzene, bromobenzene, biphenyl, p,p'-DDE [2,2-bis(p-chlorophenyl)-1,1-dichloroethylene] and 2,4,5,2',5'-pentachlorobiphenyl, A and B were found to be 5.106 and 1.258, respectively, with a coefficient of determination of 0.975. It must be emphasized that this correlation is limited with respect to being representative of the organic chemicals encountered. This calibration mixture was selected largely on the basis of the $\log K_{OW}$ values reported in the literature, and the correlation is linear over five orders of magnitude of K_{OW} . To determine the accuracy of this method of estimating $\log K_{OW}$ by comparison with data reported in the literature, Veith and coworkers measured the retention time of 18 chemicals, and the standards and $\log K_{OW}$ values were calculated from the regression equation (1). More recently Garst and Wilson (1984) have used the HPLC method to determine the partition coefficient for 66 structurally diverse chemicals ranging in $\log K_{OW}$ from 0 to near 8. Veith's results indicated that $\log K_{OW}$ can be estimated to within (22.8 ± 20.0)

percent when compared with the values reported in the literature from measurements using other methods. The percent error was calculated assuming the literature value is the correct $\log K_{OW}$; these researchers had some reservations about this assumption. It should be noted that some of the greatest relative errors were observed with polar chemicals that dissociate in water (e.g., m-chlorobenzoic acid, 2,4,5-trichloro-phenol, and diphenylamine). This method has a definite advantage, since the estimation of K_{OW} can be made rapidly and relatively easily in comparison to the determination of K_{OW} by the conventional method. Furthermore, K_{OW} can be estimated for individual chemicals in complex mixtures (e.g., solid wastes) without knowing the specific chemical structure of each chemical.

Other researchers have developed high-pressure liquid chromatographic methods to determine K_{OW} (Mirrless et al. 1976; Carlson et al. 1975; Hulshoff and Perrin 1976; McCall 1975). However, these methods are based on a very limited number of experiments and considerably more work is needed to develop them.

3. Estimation from Water Solubility

By definition, the partition coefficient expresses the equilibrium ratio of an organic chemical partitioned between an organic liquid (i.e., n-octanol) and water. This partitioning is, in essence, equivalent to partitioning of the organic

chemical between itself and water. Thus, one would expect that a correlation might exist between the partition coefficient (K_{OW}) and water solubility(s). Indeed, as shown by Mackay (1977), both of these properties are a function of the aqueous phase activity coefficient of the compound, and the correlation between the K_{OW} and S is based on the ratio of the activity coefficients in water and octanol.

Chiou et al. (1977) studied the relationship between K_{OW} and the water solubility, S , and found that, for 34 organic chemicals, an excellent linear correlation was observed between $\log K_{OW}$ and $\log S$ that extended to more than eight orders of magnitude in water solubility (10^{-3} to 10^4 ppm), and six orders of magnitude in K_{OW} (10 to 10^7). From their data the following regression equation was derived:

$$\log K_{OW} = 5.00 - 0.670 \log S, r^2 = 0.970$$

where S is expressed in $\mu\text{mol/L}$, and r^2 is the coefficient of determination.

To date a total of 18 different regression equations have been derived that correlate water solubility with the octanol water partition coefficient. This large number of equations results from the fact that solubility varies with the functional group of the molecule. Lyman et al. (1982) have summarized these

equations along with the class and number of chemicals that apply to each equation, the respective correlation coefficient, the applicable range of S and K_{OW} values and the temperature at which the solubility data were obtained. Recent developments in the correlation between S and K_{OW} indicate that many of the above equations can be combined into a single general relationship (Lyman, 1984). This would greatly simplify the estimation procedure, but introduces greater error, particularly for certain classes of compounds.

This method has a definite advantage in that K_{OW} can be estimated from an experimentally determined parameter - water solubility. Although this approach also has a cost advantage in that two parameters are determined for the cost of one, it must be kept in mind that the K_{OW} is an estimated value subject to the limitations usually associated with approximations. Thus, while the estimation of K_{OW} from water solubility is definitely a valid method, the values obtained cannot be construed as being equivalent to those obtained from the conventional shake-flask method, the HPLC method, or the generator column method.

4. Estimation Using the Fragment Constant Method

Hansch and Leo (1979) have developed a method to estimate K_{OW} from empirically derived atomic or group fragment constants

(f) and structural factors (F). Using these, the $\log K_{ow}$ is calculated using the following equation:

$$\log K_{ow} = \text{Sum of fragments (f) + structural factors (F)}$$

Of course, the critical piece of information required to apply this method is the structure of the chemical in question--which is not always known. However, if the structure is known then there are a total of over 200 fragments (f and F) available that take into account such structural factors as molecular flexibility, unsaturation, multiple halogenation, branching, and h-polar fragment interactions. These and the method of calculation, with examples, are reviewed extensively by Lyman et al. (1982). In addition, a large collection (about 15,000) of both measured and calculated K_{ow} values has been compiled by Hansch and Leo (1979); the method is also available for use on computer (Chou and Jurs 1979).

Estimation by the fragment constant is probably the best initial step in determining K_{ow} since it can be done without experimentation and is quite reliable for a large number of common organic chemicals. However, for some functional groups and complex or highly substituted molecules the fragment constant method can give erroneous, misleading results.

5. Estimation Using Thin-Layer Chromatography

It has been reported that thin-layer chromatography can be used to estimate K_{OW} (Mirrless et al. 1976; Hulshoff and Perrin 1976). However, the generator column method is far superior to thin-layer chromatography (TLC) because of its accuracy, i.e., definition of the peak, reproducibility; ease of detection in many cases; and above all the range of applicability: the generator column method is applicable over 5 orders of magnitude of K_{OW} while TLC is only applicable over 1.5 orders of magnitude of K_{OW} (Mirrless et al. 1976).

C. Rationale for the Test Conditions

1. Special Laboratory Equipment

For the determination of the molar concentration of the test substance in water, several pieces of special laboratory equipment are required. This equipment is the same as that used in the generator column method for water solubility, CG-1510 (EPA, 1983) and includes: 1) a specially designed generator column; 2) a constant temperature bath; 3) a high pressure liquid chromatograph with detector and integrator; 4) a specially designed extractor column; 5) two high pressure rotary switching valves; 6) specially designed collection vessels; and 7) a gas chromatograph with detector. In addition, particular types or brand names of column packing are specified. All of the special

laboratory equipment is same as that used by Tewari et al (1982) who developed and validated the test method. Thus, in order to maintain the integrity of the test method the procedure should be conducted using the special equipment as described. Any changes in or modification to the equipment should be reported in detail.

2. Temperature Control

From the theory of the distribution law as outlined in the test support document for determining K_{ow} by the conventional shake flask method, CG-1400 (EPA, 1983), the distribution coefficient K_{ow} is a function of the temperature, and is a constant at a fixed temperature. Hence, in performing the octanol water partition coefficient measurements using the generator column, the temperature should be controlled. Controlling the temperature to ± 0.05 °C is easy to carry out and is inexpensive.

3. Purity of Octanol, Water and Other Solvents

Dissolved salts and other impurities can affect the solubility of a compound in octanol, water or any solvent. Thus, the test guideline specifies the use of purified n-octanol and reagent grade water. Trace amounts of impurities present in n-octanol tend to produce emulsions and must be removed. The purified n-octanol may either be prepared according to the procedure given in the guideline or purchased from Fisher

Scientific. The water is prepared according to ASTM 01193-77: Standard Specification for Water. All other solvents used in the test method should be reagent or HPLC grade and contain no impurities that could interfere with the determination of the test compound.

D. Test Data and Reporting

1. Test Report

As a matter of standard laboratory practice, three determinations of the K_{ow} for each test substance are required along with the mean and standard deviation. In addition, the weights of test substance and octanol used in the preparation of the test solution are required, as is the molar concentration of the test solution. Other ancillary data required for a complete assessment of the results include: test temperature, and if the HPLC method is employed, the method used to determine the sample loop volume and its average and standard deviation from three runs, and the response factor and its mean and standard deviation. For the GC method, data on the calibration procedure, the resulting regression equation, and results from the replicate GC runs should be reported in order that the results can be validated.

2. Analytical Procedures

In those cases where a separate analytical procedure is used to determine the concentration of the test substance in n-octanol a description of the analytical method should be given in the test report. This will enable the accuracy and precision of the gravimetric method for preparing the test substance in n-octanol to be assessed. If any changes are made or problems encountered in the test method, they should be reported so that any necessary revisions can be incorporated in the test method.

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