

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Adopted by the Council on 27th July 1995

Partition Coefficient (n-octanol/water): Shake Flask Method

INTRODUCTION

1. This guideline is a revised version of the original Guideline 107 which was adopted in 1981. The only change is one of format. The substantive content of the original guideline was not changed.

INITIAL CONSIDERATIONS

2. P_{ow} values in the range $\log P_{ow}$ between -2 and 4 (occasionally up to 5) can be experimentally determined by the method set out in this guideline (1)(2). P_{ow} values in the range $\log P_{ow}$ between 0 and 6 can be estimated using high performance liquid chromatography as is set out in Guideline 117. Before deciding on what procedure to use, a preliminary estimate of the P_{ow} should be obtained from calculation (see the annex to Guideline 117), or where appropriate from the ratio of the solubilities of the test substance in the pure solvents (3).

3. Before determining P_{ow} it is useful to have preliminary information on the structural formula, dissociation constant, water solubility, hydrolysis, n-octanol solubility and surface tension of the substance.

4. The shake flask method is impossible to use with surface-active materials (for these, a P_{ow} can be calculated from individual solubilities in water and n-octanol).

DEFINITIONS AND UNITS

5. The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$P_{ow} = \frac{C_{n-octanol}}{C_{water}}$$

The partition coefficient, being the quotient of two concentrations, or the quotient of the fractions of the test substance in the two phases multiplied by a fixed volume ratio, is dimensionless and is usually given in the form of its logarithm to base ten.

REFERENCE SUBSTANCES

6. Reference substances do not need to be employed. They serve primarily to check the performance of the method from time to time and to allow comparison with results from other methods.

PRINCIPLE OF THE METHOD

7. The Nernst partition law applies at constant temperature, pressure and pH for dilute solutions. It strictly applies to a pure substance dispersed between two pure solvents and when the concentration of the solute in either phase is not more than 0.01 mol per litre. If several different solutes occur in one or both phases at the same time, this may affect the results. Dissociation or association of the dissolved molecules result in deviations from the partition law. Such deviations are indicated by the fact that the partition coefficient becomes dependent upon the concentration. Measurements should be made on ionizable substances only in their non-ionized form (free acid or free base) produced by the use of an appropriate buffer with a pH of at least one unit below (free acid) or above (free base) the pK.

DESCRIPTION OF THE METHOD**Test substance and solvents**

8. n-Octanol of analytical grade and distilled or double-distilled water should be used. Water taken directly from an ion exchanger should not be used. A stock solution of known concentration of the test substance in n-octanol, pre-saturated with water, is prepared. This solution should be stored under conditions which ensure its stability.

9. Before a partition coefficient is determined, the two solvents are mutually saturated at the temperature of the experiment. To do this, it is practical to shake two large stock bottles, one containing n-octanol and a sufficient quantity of water, and the other containing water and a sufficient quantity of n-octanol, for 24 hours on a mechanical shaker, and then to let them stand long enough to allow the phases to separate.

Test conditions

10. The test should be done at a temperature in the range 20 to 25°C, kept constant at $\pm 1^\circ\text{C}$.

11. For a first run, a volume ratio of n-octanol to water and a quantity of substance are chosen with regard given to:

- the preliminary estimation of the partition coefficient,
- the minimum concentration of test substance in each phase required for the analytical procedure,
- a maximum concentration of the test substance in each phase of 0,01 mol per litre.

For a second run, the originally chosen volume ratio is divided by two, and, for a third run, it is multiplied by two. In the second and third run the quantity of substance added may have to be different from that used in the first run in order to fulfill the above criteria.

12. Duplicate vessels containing accurately measured amounts of the two solvents and stock solution are used in all three runs.

Establishment of the partition equilibrium

13. The two-phase system should nearly fill the entire volume of the test vessels. This will help prevent loss of material due to volatilisation.

14. The test vessels are placed in a mechanical shaker or are shaken by hand. When using a centrifuge tube as the test vessel, it is recommended to rotate the tube through 180° about its transverse axis (approximately a hundred times during five minutes), allowing the trapped air to rise through the two phases.

Phase separation

15. The separation of the two phases, in general, is achieved by centrifugation. This should preferably be done at the test temperature. If a centrifuge without temperature control is used, the centrifuge tubes should be kept for equilibration at the test temperature for at least one hour before analysis.

Analysis

16. It is necessary to determine the concentrations of the test substance in both phases. For the measurement, substance-specific methods are preferred. Analytical methods which may be appropriate are: photometry, gas chromatography and high performance liquid chromatography. The total quantity of substance present in both phases should be calculated and compared with the quantity originally introduced.

17. The aqueous phase should be sampled by a procedure that minimizes the risk of including traces of n-octanol. This can be done using a syringe with a removable needle. The syringe should initially be partially filled with air. Air should be gently expelled while passing the n-octanol layer. The adequate volume of the aqueous solution is withdrawn. The syringe is quickly withdrawn and the needle is detached.

DATA AND REPORTING

Data

18. A P_{OW} value is calculated from the data of each run. Altogether six values are obtained since the test conditions are: three duplicate runs with different solvent ratios, and possibly also different quantities of test substance. The six log P_{OW} values should fall within a range of ± 0.3 units.

Test Report

19. The test report must include the following information:

- chemical identity and impurities;
- the results of the preliminary estimation (when the shake flask method is not applicable, e.g. surface active material, a calculated value or an estimate based on the individual n-octanol and water solubilities should be provided);
- all information relevant for the interpretation of the results, especially with regard to impurities and physical state of the substance;
- the test conditions: temperature, the amount of test substance introduced in the test vessels, the volume of each phase in each vessel and the calculated total amount of test substance based on the analytical data;
- pH of the water used and of the aqueous phase during the experiment;

- justification for the use of buffers; composition, concentration and pH of the buffers; pH of the aqueous phase before and after the experiment;
- duration and speed of centrifugation, if used;
- the analytical procedures;
- the concentrations measured in each run (a total of 12 concentrations);
- P_{OW} values and their mean for each set of test conditions and the overall mean (if there is the suggestion of concentration dependence of the partition coefficient, this should be noted);
- the standard deviation of individual P_{OW} values about their mean;
- the overall mean expressed as its logarithm to base 10;
- the theoretical P_{OW} when it has been calculated or when the measured value is above 10^4 .

LITERATURE

- (1) NF T 20-043 AFNOR (1985). Chemical products for industrial use - Determination of partition coefficient - Shake flask method
- (2) 40 CFR, 796.1550, Office of the Federal Register (1989)
- (3) Jübermann, O. (1958). in Houben-Weyl, Methoden der Organischen Chemie, Band I/1, 223-339, Georg Thieme Verlag, Stuttgart