C.7. DEGRADATION -ABIOTIC DEGRADATION HYDROLYSIS AS A FUNCTION OF pH

1. METHOD

This method is based on the OECD Test Guideline (1).

1.1. INTRODUCTION

Hydrolysis is an important reaction controlling abiotic degradation. This reaction is particularly relevant for substances with low biodegradability; and it can influence the persistence of a substance in the environment.

Most hydrolysis reactions are of pseudo first-order and, therefore, half-life times are independent of concentration. This usually allows the extrapolation of results found at laboratory concentration to environmental conditions.

Furthermore, several examples have been reported (2), showing a satisfactory agreement between the results found in pure and natural waters for several types of chemicals.

It is useful to have preliminary information on the vapour pressure of the substance to perform this test.

This method is applicable only to water-soluble substances. Impurities may affect the results.

Hydrolytic behaviour of chemicals should be examined at pH values more commonly found in the environment (pH 4 to 9).

1.2. DEFINITIONS AND UNITS

Hydrolysis refers to a reaction of a chemical RX with water. This reaction may be represented by the net exchange of the group X with OH:

$$RX + HOH \rightarrow ROH + HX \qquad [1]$$

The rate at which the concentration of RX decreases is given by:

$$\text{rate} = k\,[H_2O]\,.[RX] \qquad [2]$$

Because water is present in great excess compared to the chemical, this type of reaction is usually described as a pseudo-first order reaction in which the observed rate constant is given by the relationship:

$$k_{obs} = k \times [H_2O] \qquad [3]$$

This constant can be determined for one pH value and one temperature, T, using the expression:

$$k_{obs} = \frac{2.303}{t} \times \log \frac{C_o}{C_t} \qquad [4]$$

where:

t = time,

C_o = the concentration of the substance at time 0,

C_t = the concentration of the substance at time t, and

2,303 = the conversion factor between natural and base 10 logarithms.

The concentrations are expressed in grams per litre or moles per litre.

The dimension of this constant k_{obs} is $(\text{time})^{-1}$.

'The half-life period' $t_{1/2}$, is defined as the time required to reduce the concentration of the test substance by 50%, that is:

$$C_t = 1/2.C_o \quad [5]$$

From the expressions (4) and (5) one can demonstrate that:

$$t_{1/2} = 0{,}693/k_{obs} \quad [6]$$

1.3. REFERENCE SUBSTANCES

It is not necessary to use reference substances in all cases when investigating a new substance. They should serve primarily to check the performance of the method from time to time and to allow comparison with results from another method.

The following substances have been used as reference substances (1):

Acetylsalicylic acid (aspirin)

Phosphorothioic acid O,O-diethyl 0-(6-methyl-2-(1-methylethyl)4-pyrimidinyl) ester. (Dimpylate, Diazinon)

1.4. PRINCIPLE OF T HE TEST METHOD

The substance is dissolved in water at a low concentration; the pH and the temperature are controlled.

The decrease of the concentration of the substance with time is followed by any suitable analytical procedure.

The logarithm of the concentration is plotted against time and, if the plot is a straight line, the first-order rate constant may be obtained from its slope (see point 2).

When it is not practical to determine a rate constant directly for a particular temperature, it is usually possible to estimate the constant through the use of the Arrhenius relationship, which gives the temperature dependence of the rate constant. From the linear plot of the logarithm of the rate constant, as determined at appropriate temperature, as a function of the reciprocal of the absolute temperature, K, it is possible to extrapolate the rate constant value which was not directly obtainable.

1.5. QUALITY CRITERIA

It is reported in reference (2) that measurements of hydrolysis rate-constants on 13 classes of organic structures can be of high precision.

The repeatability depends in particular on the control of the pH and the temperature and might be affected by the presence of micro-organisms and in special cases by the dissolved oxygen concentration.

1.6. DESCRIPTION OF THE TEST METHOD

1.6.1. Reagents

1.6.1.1. Buffer solutions

The test is carried out at three pH values: 4,0, 7,0 and 9,0.

For this purpose, buffer solutions should be prepared using reagent grade chemicals and distilled or deionized, sterile water. Some examples of buffer systems are presented in the Appendix.

The buffer system used may influence the rate of hydrolysis; if there is evidence of this, an alternative buffer system should be employed. The use of borate or acetate buffers is recommended in reference (2) instead of phosphate.

If the pH value of the buffer solutions is not known at the temperature used during the test, this can be determined with a calibrated pH meter at the selected temperature with a precision of ± 0,1 pH units.

1.6.1.2. Test solutions

The test substance should be dissolved in the selected buffer and the concentration should not exceed 0,01 M or half the saturation concentration, whichever is the lower.

The use of water-miscible organic solvents is recommended only for substances of low water solubility.

The amount of solvent should be less than 1% , and should not interfere with the hydrolytic process.

1.6.2. Apparatus

Stoppered glass flasks should be used, but grease must be avoided on the ground joint.

If the chemical or the buffer system is volatile, or if the test is being conducted at elevated temperatures, sealed or septum-closed tubes are preferred and head space should be avoided.

1.6.3. Analytical method

The method must be specific to allow determination of the test substance at the test solution concentrations and may well consist of some combination of suitable analytical techniques.

The analytical method used will depend on the nature of the substance and must be sufficiently precise and sensitive to detect a reduction of 10% of the initial concentration.

1.6.4. Test conditions

The tests will be carried out using a thermostatically controlled enclosure or a constant-temperature bath set at ± 0,5 °C of the chosen temperature. The temperature will be kept and measured to within ± 0,1 °C. Photolytic interference should be avoided by appropriate means.

For substances which are easily oxidizable, it will be necessary to exclude dissolved oxygen (e.g. by bubbling with nitrogen or argon for five minutes before preparation of the solution).

1.6.5. Test Procedure

1.6.5.1. Preliminary test

For all substances a preliminary test should be performed at 50 ± 0,5 °C at three pH values: 4,0, 7,0 and 9,0. A sufficient number of measurements are made, in order to be able to estimate whether, for each pH value and at 50 °C, the half-life time ($t_{1/2}$) is lower than 2,4 hours or less than 10% of hydrolysis is observed after five days. (One can estimate that these values correspond respectively to half-life times lower than one day or higher than one year under conditions more representative of those of the environment (25 °C)). If the preliminary test indicates that 50% or more of the test substance has been hydrolyzed in 2,4 hours at 50 °C, or less than 10 % has been hydrolyzed after five days at each of the three pH values (4, 7 and 9), no further testing is necessary.

In other cases, and for individual pH values for which this condition has not been fulfilled, test 1 is carried out.

1.6.5.2. Test 1

Test 1 is carried out at one temperature; preferably at 50 ± 0,5 °C, and, if possible, under sterile conditions at those pH values for which the preliminary test has shown the necessity for further testing.

A sufficient number of samples (not less than four) should be chosen to cover the range 20 to 70% of hydrolysis to test for pseudo-first order behaviour at the specified pH values.

For each pH value at which test 1 is performed the order of reaction is determined.

Estimation of rate constant at 25 °C:

The decision on how to proceed experimentally depends on whether it may be concluded from test 1 that the reaction is pseudo-first order or not.

If it cannot be concluded with certainty from test 1 that the reaction is pseudo-first order, further experiments ,must be carried out as described in test 2.

If it can be safely concluded from test 1 that the reaction is pseudo-first order, further experiments should be carried out as described in test 3. (Alternatively, it may, under special circumstances, be possible to calculate the rate constants at 25 °C from constants at 50 °C, calculated using the results from test 1, (see 3.2)).

1.6.5.3. Test 2

This test is performed, at each pH value for which the results of test 1 have shown the necessity to do so:

-either at one temperature lower than 40 °C,

-or at two temperatures above 50 °C differing from each other by at least 10 °C.

For each pH value and temperature where test 2 is carried out, at least six adequately spaced data points should be taken so that the degrees of hydrolysis are in the range 20 to 70%.

For one pH value and one temperature, a determination is carried out in duplicate. When test 2 is done at two temperatures above 50 °C, the duplicate is preferably performed at the lower of these two temperatures.

For each pH value and temperature where test 2 is carried out, a graphical estimation of the half-life time ($t_{1/2}$) will be given when possible.

1.6.5.4. Test 3

This test is carried out, at each pH value for which the results of test 1 have shown the necessity to do so.

-either at one temperature lower than 40 °C,

-or at two temperatures above 50 °C differing from each other by at least 10 °C.

For each pH and temperature where test 3 is performed, three data points are chosen, the first at time 0 and the second and third when the degree of hydrolysis is greater than 30%; the constant k_{obs} and $t_{1/2}$ should be calculated.

2. **DATA**

For pseudo-first order behaviour the values of k_{obs} for each pH value and each temperature of the tests can be obtained from the plots of the logarithms of the concentration versus time using the expression:

$$k_{obs} = -\text{slope} \times 2{,}303 \qquad [7]$$

Furthermore $t_{1/2}$ can be calculated according to equation [6].

Evaluate $k_{25\,°C}$ by applying the Arrhenius equation where appropriate.

For non-pseudo-first order behaviour see 3.1.

3. **REPORT**

3.1 REPORTING

The test report shall, if possible, include the following information:

-specification of the substance;

-any results obtained with reference substances;

-the principle and details of the analytical method used;

-for each test: the temperature, pH value, buffer composition and a table of all concentration-time data points;

-for pseudo-first order reaction, the values of k_{obs} of $t_{1/2}$ and its calculation procedure;

-for non-pseudo-first order reaction, plot results as logarithm of concentration versus time;

-all information and observations necessary for the interpretation of the results.

3.2. INTERPRETATION OF RESULTS

It may be possible to calculate acceptable values of the rate constant (at 25 °C) of test substances, provided that experimental values of the activation energy already exist for homologues of the test substance and provided that it can be reasonably assumed that the activation energy of the test substance is of the same order of magnitude.

4. **REFERENCES**

(1) OECD, Paris, 1981, Test Guideline 111, Decision of the Council C(81) 30 final.

(2) W. Mabey and T. Mill, 'Critical Review of Hydrolysis of Organic Compounds in Water Under Environmental Conditions,' J. Phys. Chem. Ref. Data, 1978, vol. 7 (2),383-415.

Appendix

BUFFER MIXTURES

A. CLARK AND LUBS

The pH values reported in these tables have been calculated from the potential measurements using Sorensen standard equations. The actual pH value are 0,04 unit higher than the tabulated values.

Composition	pH
0,1 M potassium hydrogen phthalate + 0,1 N HCI at 20 °C	
2,63 ml 0,1 N HCI + 50 ml phthalate to 100 ml	3,8
0,1 M potassium hydrogen phthalate + 0,1 N NaOH at 20 °C	
0,40 ml 0,1 N NaOH + 50 ml phthalate to 100 ml	4,0
3,70 ml 0,1 N NaOH + 50 ml phthalate to 100 ml	4,2
0,1 M monopotassium phosphate + 0,1 N NaOH at 20 °C	6,8
29,63 ml 0,1 N NaOH + 50 ml phosphate to 100 ml	7,0
0,1 M H_3BO_3 in 0,1 M KCI + 0,1 N NaOH at 20 °C	
16,30 ml 0,1 N NaOH + 50 ml boric acid to 100 ml	8,8
21,30 ml 0,1 N NaOH + 50 ml boric acid to 100 ml	9,0
26,70 ml 0,1 N NaOH + 50 ml boric acid to 100 ml	9,2

B. KOLTHOFF AND VLEESHOUWER

Composition	pH
0,1 M monopotassium citrate and 0,1 N NaOH at 18 °C (add tiny crystal of thymol to prevent growth of moulds)	

2,0 ml 0,1 N NaOH + 50 ml citrate to 100 ml 3,8

9,0 ml 0,1 N NaOH + 50 ml citrate to 100 ml 4,0

16,3 ml 0,1 N NaOH + 50 ml citrate to 100 ml 4,2

C. SÖRENSEN

0,05 M borax + 0,1 N HCI

Composition		pH			
			Walbum		
ml Borax	ml HCl	Sörensen 18 °C	10 °C	40 °C	70 °C
8,0	2,0	8,91	8,96	8,77	8,59
8,5	1,5	9,01	9,06	8,86	8,67
9,0	1,0	9,09	9,14	8,94	8,74
9,5	0,5	9,17	9,22	9,01	8,80
10,0	0,0	9,24	9,30	9,08	8,86

0,05 M borax + 0,1 N NaOH

Composition		pH			
			Walbum		
ml Borax	ml NaOH	Sörensen 18 °C	10 °C	40 °C	70 °C
10,0	0,0	9,24	9,30	9,08	8,86
9,0	1,0	9,36	9,42	9,18	8,94
8,0	2,0	9,50	9,57	9,30	9,02
7,0	3,0	9,68	9,76	9,44	9,12