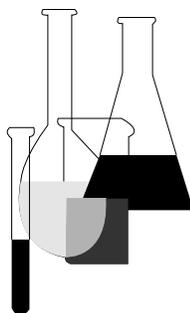




Product Properties Test Guidelines

OPPTS 830.7370

Dissociation Constants in Water



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 830.7370 Dissociation constants in water.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are the OPPT guideline under 40 CFR 796.1370 Dissociation constants in water, OPP guideline 63–10 Dissociation Constant (Pesticide Assessment Guidelines, Subdivision D: Product Chemistry, EPA Report 540/9–82–018, October 1982) and OECD guideline 112 Dissociation Constants in Water.

(b) **Introductory information.** (Titration method; spectrophotometric method; conductometric method)

(1) **Prerequisites.** (i) Suitable analytical method.

(ii) Water solubility.

(2) **Guidance information.** (i) Structural formula.

(ii) Electrical conductivity for conductometric method.

(3) **Qualifying statements.** (i) All test methods may be carried out on pure or commercial grade substances. The possible effects of impurities on results should be considered.

(ii) The titration method is not suitable for low solubility compounds (see test solutions, under paragraph (c)(2)(ii)(A) of this guideline).

(iii) The spectrophotometric method is only applicable to compounds having appreciably different UV/VIS absorption spectra for the dissociated and undissociated forms. This method may also be suitable for low solubility compounds and for non-acid/base dissociations, e.g. complex formation.

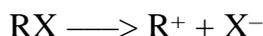
(iv) In cases where the Onsager equation holds, the conductometric method may be used, even at moderately low concentrations and even in cases for non-acid/base equilibria.

(4) **Standard documents.** This test guideline is based on methods given in the references listed in paragraph (e) of this guideline and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

(c) **Method**—(1) **Introduction, purpose, scope, relevance, application and limits of test**—(i) **Dissociation of chemicals in water.** The dissociation of a chemical in water is of importance in assessing its impact upon the environment. It governs the form of the substance which in turn

determines its behavior and transport. It may affect the adsorption of the chemical on soils and sediments and absorption into biological cells.

(ii) **Definitions and units.** *Dissociation* is the reversible splitting into two or more chemical species which may be ionic. The process is indicated generally by



and the concentration equilibrium constant governing the reaction is

$$K = [R^+] [X^-]/[RX]$$

For example, in the particular case where R is hydrogen (the substance is an acid), the constant is

$$K_a = [H^+][X^-]/[HX]$$

or

$$pK_a = pH - \log [X^-]/[HX]$$

(iii) **Reference substances.** (A) The following reference compounds need not be employed in all cases when investigating a new substance. They are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied.

		pK _a (1)
<i>p</i> -Nitrophenol	7.15	25 ¹
Benzoic acid	4.19	20
<i>p</i> -Chloroaniline	4.15	20

¹ No value is available for 20 °C, but it can be assumed that the variability of measurement results is higher than the temperature dependence to be expected.

(B) It would be useful to have a substance with several pK's as indicated in Principle of the Method, under paragraph (c)(1)(iii) of this guideline. Such a compound could be:

		pK _a (8)
Citric acid	(1) 3.14	20
	(2) 4.77	20
	(3) 6.39	20

(iv) **Principle of the test method.** The chemical process described is generally only slightly temperature dependent in the environmentally relevant temperature range. The determination of the dissociation constant requires a measure of the concentrations of the dissociated and undissociated forms of the chemical substance. From a knowledge of the stoichiometry of the dissociation reaction indicated in paragraph (c)(1)(ii)

of this guideline, the appropriate constant can be determined. In the particular case described in this guideline the substance is behaving as an acid or a base, and the determination is most conveniently done by determining the relative concentrations of ionized and un-ionized forms of the substance and the pH of the solution. The relationship between these terms is given in the equation for pK_a in paragraph (c)(1)(ii) of this guideline. Some compounds exhibit more than one dissociation constant and similar equations can be developed. Some of the methods described herein are also suitable for non-acid/base dissociation.

(v) **Quality criteria**—(A) **Repeatability.** The dissociation constant should be replicated (a minimum of three determinations) to within ± 0.1 log units.

(B) [Reserved]

(2) **Description of the test procedures**—(i) **Determination approaches.** There are two basic approaches to the determination of pK_a . One involves titrating a known amount of substance with standard acid or base, as appropriate; the other involves determining the relative concentrations of the ionized and un-ionized forms and their pH dependence.

(ii) **Preparations.** Methods based on those principles may be classified as titration, spectrophotometric, and conductometric procedures.

(A) **Test solutions.** (1) For the titration method and the conductometric method the chemical substance should be dissolved in distilled water. For spectrophotometric and other methods buffer solutions are used. The concentrations of the test substances should not exceed the lesser of 0.01 M or half the saturation concentration, and the purest available form of the substance should be employed in making up the solutions. If the substance is only sparingly soluble, it may be dissolved in a small amount of a water-miscible solvent prior to adding to the concentrations indicated above.

(2) Solutions should be checked for the presence of emulsions using a Tyndall beam, especially if a co-solvent has been used to enhance solubility. Where buffer solutions are used, the buffer concentration should not exceed 0.05 M.

(B) [Reserved]

(iii) **Test conditions**—(A) **Temperature.** (1) The temperature should be controlled to at least ± 1 °C. The determination should preferably be carried out at 20 °C.

(2) If a significant temperature dependence is suspected, the determination should be carried out at two or more different temperatures. The temperature intervals should be 10 °C in this case and the temperature control ± 0.1 °C.

(B) **Analyses.** The method will be determined by the nature of the substance being tested. It must be sufficiently sensitive to allow determination of the different species at the test solution concentrations.

(iv) **Performance of the test—(A) Titration method.** The test solution is determined by titration with the standard base or acid solution as appropriate, measuring the pH after each addition of titrant. At least 10 incremental additions should be made before the equivalence point. If equilibrium is reached sufficiently rapidly, a recording potentiometer may be used. For this method both the total quantity of substance and its concentration need to be accurately known. Precautions must be taken to exclude carbon dioxide. Details of procedure, precautions, and calculation are given in standard tests, e.g., references under paragraphs (e)(1) through (e)(4) of this guideline.

(B) **Spectrophotometric methods.** A wavelength is found where the ionized and un-ionized forms of the compound have appreciably different extinction coefficients. The UV/VIS absorption spectrum is obtained from solutions of constant concentration under a pH condition where the substance is essentially un-ionized and fully ionized and at several intermediate pH's. This may be done either by adding increments of concentrated acid (base) to a relatively large volume of a solution of the compound in a multicomponent buffer, initially at high (low) pH under paragraph (e)(5) of this guideline, or by adding equal volumes of a stock solution of the compound in, e.g., water, methanol, to constant volumes of various buffer solutions covering the desired pH range. From the pH and absorbance values at the chosen wavelength, a sufficient number of values for the pK_a are calculated using data from at least five pH's where the compound is at least 10 percent and less than 90 percent ionized. Further experimental details and method of calculation are given in reference under paragraph (e)(1) of this guideline.

(C) **Conductometric method.** Using a cell of small, known cell constant, the conductivity of an approximately 0.1 M solution of the compound in conductivity water is measured. The conductivities of a number of accurately made dilutions of this solution are also measured. (The concentration is halved each time, and the series should cover at least an order of magnitude in concentration.) The limiting conductivity at infinite dilution is found by carrying out a similar experiment with the sodium salt and extrapolating. The degree of dissociation may then be calculated from the conductivity of each solution using the Onsager equation, and hence using the Ostwald dilution law the dissociation constant may be calculated as

$$K = \alpha_2 C / (1 - \alpha)$$

where C is the concentration in moles per liter and α is the fraction dissociated. Precautions must be taken to exclude CO_2 . Further experimental details and method of calculation are given in standard texts and references under paragraphs (e)(1), (e)(6), and (e)(7) of this guideline.

(d) **Data and reporting**—(1) **Treatment of results**—(i) **Titration method.** The pK_a is calculated for 10 measured points on the titration curve and the mean and standard deviation of these pK_a values are calculated. A plot of pH versus volume of standard base or acid should be included along with a tabular presentation.

(ii) **Spectrophotometric methods.** The absorbance and pH are tabulated from each spectrum. At least five values for the pK_a are calculated from the intermediate spectra data points, and the mean and standard deviation of these results are also calculated.

(iii) **Conductometric method.** The equivalent conductance, Λ , is calculated for each acid concentration and for each concentration of a mixture of one equivalent of acid, plus 0.98 equivalent of carbonate-free sodium hydroxide. (The presence of excess acid is to prevent an excess of OH^- due to hydrolysis.) Values of $1/\Lambda$ are plotted against \sqrt{C} and Λ_0 of the salt can be found by extrapolation to zero concentration. Λ_0 of the acid can be calculated using literature values for H^+ and Na^+ . The pK_a can be calculated from $\alpha = \Lambda_i/\Lambda_0$ and $K_a = \alpha^2 C / (1 - \alpha)$ for each concentration. Better values for K_a can be obtained by making corrections for mobility and activity. The mean and standard deviations of the pK_a values should be calculated.

(2) **Test report.** (i) All raw data and calculated pK_a values should be submitted together with method of calculation (preferably in a tabulated format, such as suggested in paragraph (e)(1) of this guideline) as should the statistical parameters described under paragraph (d)(1) of this guideline. For titration methods, details of the standardization of titrants should be given.

(ii) For spectrophotometric methods, all spectra should be submitted. For the conductometric method, details of the cell constant determination should be reported. Information on technique used, analytical methods, and the nature of any buffers used should be given.

(iii) The test temperatures should be reported.

(e) **References.** The following references should be consulted for additional background material on this test guideline:

(1) Albert, A. and Sergeant, E.P., *Ionization Constants of Acids and Bases*. Wiley, Inc., New York (1962).

(2) Nelson, N.H. and Faust, S.D., "Acidic Dissociation Constants of Selected Aquatic Herbicides," *Environment Science Technology* 3: II pp. 1186-1188 (1969).

(3) American Society for Testing and Materials, D-1293, ASTM, 1916 Race St., Philadelphia, PA 19103 (latest annual index).

(4) American Public Health Association, *Standard Methods for the Examination of Water and Waste Water*, Standard Method 242, 14th ed., American Public Health Association: Washington, DC (1976).

(5) Clark, J. & Cunliffe, A.E., "Rapid Spectrophotometric Measurement of Ionisation Constants in Aqueous Solution," *Chemical Index* (London 1973).

(6) American Society for Testing and Materials, D-1125, ASTM, 1916 Race St. Philadelphia, PA 19103 (latest annual index).

(7) American Public Health Association, *Standard Methods for the Examination of Water and Waste Water*. Standard Method 205, 14th ed., American Public Health Association: Washington, DC (1976).

(8) *Handbook of Chemistry and Physics*, 60th ed., CRC-Press: Boca Raton, FL (1980).

(9) *Organization for Economic Cooperation and Development*, Guidelines for The Testing of Chemicals, OECD 112 Dissociation Constants in Water, OECD, Paris, France.