



"Dissociation Constants in Water"
(Titration Method - Spectrophotometric Method -
Conductometric Method)

1. INTRODUCTORY INFORMATION

- Prerequisites

- Suitable analytical method
- Water solubility

- Guidance information

- Structural formula
- Electrical conductivity for conductometric method

- Qualifying statements

- All test methods may be carried out on pure or commercial grade substances. The possible effects of impurities on results should be considered.
- The titration method is not suitable for low solubility compounds (see Test solutions, below).
- 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.
- 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.

- Standard documents

This Test Guideline is based on methods given in the references listed in Section 4 and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

2. M E T H O D**A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE,
APPLICATION AND LIMITS OF TEST**

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 behaviour and transport. It may affect the adsorption of the chemical on soils and sediments and adsorption into biological cells.

• D e f i n i t i o n s a n d u n i t s

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

$$K = \frac{[R^+][X^-]}{[RX]}$$

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

$$K_a = [H^+] \cdot \frac{[X^-]}{[HX]}$$

or

$$pK_a = pH - \log \frac{[X^-]}{[HX]}$$

• R e f e r e n c e s u b s t a n c e s

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.

"Dissociation Constants in Water"

	pK_a (1)	<u>Temp. in °C</u>
– p-Nitrophenol	7.15	25*
– Benzoic acid	4.12	20
– p-Chloroaniline	3.93	20

It would be useful to have a substance with several pK 's as indicated in Principle of the method, below. Such a compound could be:

– Citric acid	pK_a (8)	<u>Temp. in °C</u>
	1) 3.14	20
	2) 4.77	20
	3) 6.39	20

• 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 Definitions and units, above, 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 ionised and unionised forms of the substance and the pH of the solution. The relationship between these terms is given in the equation for pK_a in Definitions and units, above. 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.

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

- Quality criteria

Repeatability

The dissociation constant should be replicated (a minimum of three determinations) to within ± 0.1 log units.

B. DESCRIPTION OF THE TEST PROCEDURES

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 ionised and unionised forms and their pH dependence.

- Preparations

Methods based on those principles may be classified as titration, spectrophotometric and conductometric procedures.

Test solutions

For the titration method and 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.

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.

- Test conditions

Temperature

The temperature should be controlled to at least $\pm 1^\circ\text{C}$. The determination should preferably be carried out at 20°C .

"Dissociation Constants in Water"

If a significant temperature dependence is suspected, the determination should be carried out at at least two other temperatures. The temperature intervals should be 10°C in this case and the temperature control $\pm 0.1^\circ\text{C}$.

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.

• Performance of the test

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 (1), (2), (3), (4).

Spectrophotometric methods

A wavelength is found where the ionised and unionised 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 unionised and fully ionised 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 (ref. 5), 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 is calculated using data from at least 5 pH's where the compound is at least 10 per cent and less than 90 per cent ionised. Further experimental details and method of calculation are given in reference (1).

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 Na 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 litre and α is the fraction dissociated. Precautions must be taken to exclude CO₂. Further experimental details and method of calculation are given in standard texts and references (1), (6) and (7).

3. DATA AND REPORTING**• Treatment of results*****Titration method***

The pK_a is calculated for 10 measured points on the titration curve. The mean and standard deviation of such pK_a values are calculated. A plot of pH versus volume of standard base or acid should be included along with a tabular presentation.

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.

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 excess acid is to prevent an excess of OH⁻ due to hydrolysis.) $1/\Lambda$ is plotted against $\sqrt{C}$ and Λ_0 of the salt can be found by extrapolation to zero concentration.

"Dissociation Constants in Water"

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.

• Test report

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 ref. 1) as should the statistical parameters described above. For titration methods, details of the standardisation of titrants should be given.

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.

The test temperature(s) should be reported.

4. L I T E R A T U R E

1. Albert, A. & Sergeant, E.P.: *Ionization Constants of Acids and Bases*, Wiley, Inc., New York, 1962.
2. Nelson, N.H. & Faust, S.D.: Acidic dissociation constants of selected aquatic herbicides, *Env. Sci. Tech.* 3, II, pp. 1186-1188 (1969).
3. ASTM D 1293 - Annual ASTM Standards, Philadelphia, 1974.
4. Standard Method 242. APHA/AWWA/WPCF, *Standard Methods for the Examination of Water and Waste Water*, 14th Edition, American Public Health Association, Washington, D.C., 1976.
5. Clark, J. & Cunliffe, A.E.: Rapid spectrophotometric measurement of ionisation constants in aqueous solution. *Chem. Ind. (London)* 281, (March 1973).
6. ASTM D 1125 - Annual ASTM Standards, Philadelphia, 1974.
7. Standard Method 205 - APHA/AWWA/NPCF (see above).
8. *Handbook of Chemistry and Physics*, 60th ed. CRC-Press, Boca Raton, Florida, 33431 (1980).