"Hydrolysis as a Function of pH"

Composition   pH

0.1 M potassium biphthalate + 0.1 NaOH at 20°

23.85 ml. 0.1 N NaOH + 50 ml. biphthalate to 100 ml 5.0
29.95 ml. 0.1 N NaOH + 50 ml. biphthalate to 100 ml 5.2
35.45 ml. 0.1 N NaOH + 50 ml. biphthalate to 100 ml 5 4
39.85 ml. 0.1 N NaOH + 50 ml. biphthalate to 100 ml 5.6
43.00 ml. 0.1 N NaOH + 50 ml. biphthalate to 100 ml 5.8
45.45 ml. 0.1 N NaOH + 50 ml. biphthalate to 100 ml 6.0

0.1 M monopotassium phosphate + 0.1 N NaOH at 20°

5.70 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 6.0
8.60 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 6.2
12.60 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 6.4
17.80 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 6.6
23.45 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 6.8
29.63 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 7.0
35.00 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 7.2
39.50 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 7.4
42.80 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 7.6
45.20 ml. 0.1 N NaOH + 50 ml. phosphate to 100 ml 7.8
46.80 ml. 0.1 N NaOH + 50 ml phosphate to 100 ml 8.0

0.1 M H_2BO_2 in 0.1 M KCl + 0.1 N NaOH at 20°

2.61 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 7.8
3.97 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 8.0
5.90 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 8.2
8.50 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 8.4
12.00 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 8.6
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
32.00 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 9.4
36.85 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 9.6
40.80 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 9.8
43.90 ml. 0.1 N NaOH + 50 ml. boric acid to 100 ml 10.0

B. KOLTHOFF AND VLEESCHHOUWER

CITRATE BUFFERS OF KOLTHOFF AND VLEESCHHOUWER

0.1 M monopotassium citrate and 0.1 N HCl at 18°
(Add tiny crystal of thymol or a few milligrams of mercury to prevent growth of molds)

Composition	pH
49.7 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	2.2
43.4 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	2.4
36.8 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	2.6
30.2 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	2.8
23.6 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	3.0
17.2 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	3.2
10.7 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	3.4
4.2 ml. 0.1 N HCl + 50 ml. citrate to 100 ml	3.6

0.1 M monopotassium citrate and 0.1 N NaOH at 18°
(Add tiny crystal of thymol or a few milligrams of mercuric iodide to prevent growth of molds)

Composition	pH
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
23.7 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	4.4
31.5 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	4.6
39.2 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	4.8
46.7 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	5.0
54.2 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	5.2
61.0 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	5.4
68.0 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	5.6
74.4 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	5.8
81.2 ml. 0.1 N NaOH + 50 ml. citrate to 100 ml	6.0

C. SÖRENSEN

BORATE MIXTURES OF SÖRENSEN

0.05 M borax + 0.1 N HCl

Composition		Sörensen 18°	Walbum, pH at		
ml. Borax	ml. HCl		10°	40°	70°
5.25	4.75	7.62	7.64	7.55	7.47
5.50	4.50	7.94	7.98	7.86	7.76
5.75	4.25	8.14	8.17	8.06	7.95
6.00	4.00	8.29	8.32	8.19	8.08
6.50	3.50	8.51	8.54	8.40	8.28
7.00	3.00	8.08	8.72	8.56	8.40
7.50	2.50	8.80	8.84	8.67	8.50
8.00	2.00	8.91	8.96	8.77	8.59
8.50	1.50	9.01	9.06	8.86	8.67
9.00	1.00	9.09	9.14	8.94	8.74
9.50	0.50	9.17	9.22	9.01	8.80
10.0	0.00	9.24	9.30	9.08	8.86

0.05 M borax + 0.1 N NaOH

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
6.0	4.0	9.97	10.06	9.67	9.28

2. RATE CONSTANTS AND HALF-LIVES OF SOME SELECTED CHEMICALS

A. REPORTED LITERATURE VALUES ON ASPIRIN AND DIAZINON

The following literature values have been reported:

Diazinon (0, 0-ondiethyl-0- (6-methyl-2-isopropyl-4-pyrimidinyl phosphrothioate) from H.M. Gomma, I.H. Suffet, S.D. Faust, Residue Reviews 29, 171 (1969):

	10.43	0.061 (310)	0.13 (150)	0.41 (48)	15 (12)	K [10^5 sec^{-1}] ($t_{1/2}$ [h])
	9.0		0.0059 (3300)			K [10^5 sec^{-1}] ($t_{1/2}$ [h])
pH	7.4		0.00043 (4400)			K [10^5 sec^{-1}] ($t_{1/2}$ [h])
	5.0		0.026 (740)			K [10^5 sec^{-1}] ($t_{1/2}$ [h])
	3.1	0.75 (260)	1.6 (120)	6.6 (29)	25 (7.8)	K [10^5 sec^{-1}] ($t_{1/2}$ [h])
		10°C	20°C	40°C	60°C	Temperature

Aspirin (2-acetylsalicylic acid) from L.J. Edwards, Trans. Faraday Soc. 723-735 (1950)

	0.065 (300)	0.15 (130)	0.13 (150)	0.37 (52)	16 (1.2)	K [10^5 sec^{-1}] ($t_{1/2}$ [h])
pH	3.5	5.0	7.4	9.5	11.3	

at 17°C

B. RESULTS OF OECD-EEC LABORATORY INTERCOMPARISON TESTING PROGRAMME, PART 2 (4)

– Aspirin
– Diazinon

In some cases the results presented here have a variability which exceeds what is called for in the Test Guideline. This may particularly be the result of the use of different buffers in the test systems and the influence of oxygen.

The Expert Group presents these results however, as they have been obtained using the Test Guidelines in the OECD/EEC Intercomparison Testing Programme, Part II. The first Guideline as presented here has been modified in the light of these results.

Summary Results of "Hydrolysis as a function of pH"

Reaction Rate Constant in 10^5. 1/s

Substance: Aspirin

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	1.013	1.278	126.2	0.109 to 1.916	2
3.0	20	0.080	0.056	70.3	0.040 to 0.119	2
	40	0.556	0.112	20.1	0.477 to 0.635	2
	60	-	-	-	-	-
7.0	20	0.205	0.033	15.9	0.182 to 0.228	2
	40	1.339	0.004	0.3	1.336 to 1.341	2
	60	-	-	-	-	-
9.0	20	0.309	0.160	51.7	0.196 to 0.442	2
	40	0.953	0.006	0.6	0.949 to 0.957	2
	60	-	-	-	-	-

"Hydrolysis as a Function of pH"

Summary Results of "Hydrolysis as a Function of pH"

Half Life Time in Hours ($t_{1/2}$)

Substance: Aspirin

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	93.71	118.3	126	10.05 to 177.36	2
3.0	20	319.7	222.5	70	162.3 to 477.0	2
	40	35.4	7.1	20	30.3 to 40.4	2 (1 lab)
	60	-	-	-	-	-
7.0	20	95.1	15.1	20	84.4 to 105.8	2
	40	14.4	0.0	-	14.4 to 14.4	2 (1 lab)
	60	-	-	-	-	-
9.0	20	72.1	37.3	50	45.7 to 98.5	2
	40	20.2	0.1	0.0	20.1 to 20.3	2 (1 lab)
	60	-	-	-	-	-

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 10^5.1/s
Substance: Diazinon

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	30.36	8.10	27.0	21.40 to 38.46	4
3.0	20	2.866	0.825	28.8	1.675 to 3.841	7
	40	9.038	2.447	27.1	5.708 to 14.165	11 (10 labs)
	50	5.77	4.27	74.0	0.86 to 8.58	3 (2 labs)
	60	36.085	11.088	30.7	25.535 to 51.449	6
7.0	20	0.933	1.796	192.4	0.005 to 3.626	4
	40	0.231	0.294	127.3	0.042 to 0.895	8
	50	0.200	0.023	11.3	0.184 to 0.216	2
	60	1.638	3.154	192.6	0.303 to 9.413	8 (7 labs)
9.0	20	1.103	2.113	191.6	0.007 to 4.271	4
	40	2.568	6.900	268.7	0.064 to 20.955	9
	50	0.292	0.034	11.6	0.268 to 0.316	2
	60	2.801	4.125	147.3	0.241 to 12.604	8 (7 labs)

Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: Diazinon

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	0.672	0.187	27.9	0.501 to 0.900	4
3.0	20	7.27	2.40	33	5.0 to 11.5	7
	40	2.25	0.57	25	1.4 to 3.3	11 (10 labs)
	50	9.00	11.53	128	2.24 to 22.31	3 (2 labs)
	60	0.57	0.17	29	0.37 to 0.75	6
7.0	20	1707.75	1875.18	110	5.3 to 3660.9	4
	40	215.16	166.12	77	21.5 to 460.7	8
	50	97.07	10.97	11	89.31 to 104.83	2
	60	38.63	20.98	54	2.0 to 63.5	8 (7 labs)
9.0	20	1150.75	1335.58	116	4.5 to 2840.5	4
	40	124.38	102.45	82	0.9 to 298.7	9
	50	66.40	7.74	12	60.92 to 71.87	2
	60	25.13	25.67	102	1.5 to 79.8	8 (7 labs)

C. FURTHER RESULTS OF OECD-EEC LABORATORY INTERCOMPARISON TESTING PROGRAMME (4)

Three other compounds were similarly tested in this programme and the results are presented below

- Atrazine
- Diethyl-hexylphthalate
- Ethylacetate

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 10^5. l/s
Substance: Atrazine

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	0.546	0.416	76.3	0.076 to 0.948	4
3.0	20	0.014	0.008	56.8	0.005 to 0.020	3
	40	0.140	0.082	58.4	0.028 to 0.222	6
	60	0.808	0.397	49.1	0.282 to 1.283	5
7.0	20	-	-	-	-	2
	40	0.009	0.011	124.8	0.001 to 0.016	2
	60	-	-	-	-	-
9.0	20	0.013	0.016	130.1	0.001 to 0.024	-
	40	0.008	0.010	123.7	0.001 to 0.015	2
	60	-	-	-	-	-

Reaction Rate Constant in 10^5. l/s
Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: Atrazine

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	54.76	44.36	81.0	20.3 to 115.35	4
3.0	20	1867.88	1446.58	77.0	980.7 to 3537.14	3
	40	240.50	239.61	99.6	111.71 to 696.79	6
	60	31.42	21.51	69.0	15.0 to 68.3	5
7.0	20	-	-	-	-	2
	40	9000.15	11033.77	123.0	1198.1 to 16802.2	2
	60	-	-	-	-	-
9.0	20	17921.05	24188.21	135.0	817.4 to 35024.7	2
	40	8005.35	9539.51	119.0	1259.9 to 14750.8	2
	60	-	-	-	-	-

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 10^5. 1/s
Substance: DOP

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	-
3.0	20	0.048	0.051	107.8	0.009 to 0.106	3
	40	0.166	0.202	121.8	0.023 to 0.309	2
	60	0.084	0.022	26.3	0.068 to 0.099	2
7.0	20	0.073	0.064	88.8	0.027 to 0.118	2
	40	(1.627)	-	-	-	1
	60	(0.092)	-	-	-	1
9.0	20	0.040	0.047	116.7	0.007 to 0.073	2
	40	(0.126)	-	-	-	1
	60	-	-	-	-	-

Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours ($t_{1/2}$)
Substance: DOP

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	-
3.0	20	990.20	996.32	101	182.4 to 2103.5	3
	40	452.56	551.98	122	62.25 to 842.86	2
	60	239.33	63.75	27	194.25 to 284.41	2
7.0	20	440.30	391.35	89	163.57 to 717.02	2
	40	(11.83)	-	-	-	1
	60	(208.19)	-	-	-	1
9.0	20	1606.74	1898.79	118	265.51 to 2947.97	2
	40	(152.53)	-	-	-	1
	60	-	-	-	-	-

Summary Results of "Hydrolysis as a Function of pH"
Reaction Rate Constant in 105.1/s
Substance: Ethylacetate

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	1
3.0	20	(0.0012)	-	-	-	1
	40	(0.012)	-	-	-	1
	60	(0.355)	-	-	-	1
7.0	20	(0.003)	-	-	-	1
	40	(0.008)	-	-	-	1
	60	(0.137)	-	-	-	1
9.0	20	(0.063)	-	-	-	1
	40	(0.153)	-	-	-	1
	60	(1.547)	-	-	-	1

Summary Results of "Hydrolysis as a Function of pH"
Half Life Time in Hours $(t_{1/2})$
Substance: Ethylacetate

pH-value	temperature in °C	mean value	standard deviation	coefficient of variation (%)	range	n° of results
1.2	35-40	-	-	-	-	-
3.0	20	(1656)	-	-	-	1
	40	(1612.85)	-	-	-	1
	60	(54.30)	-	-	-	1
7.0	20	(7553.1)	-	-	-	1
	40	(2511.81)	-	-	-	1
	60	(140.38)	-	-	-	1
9.0	20	(305.55)	-	-	-	1
	40	(125.71)	-	-	-	1
	60	(12.44)	-	-	-	1

3. SUGGESTED FORM TO REPORT DATA

Laboratory:
Test substance:
Date:

Test Protocol

A. PRELIMINARY TEST

☐   yes   ☐   no

buffer systems used:

     pH 4.0 . . . .
     pH 7.0 . . . .
     pH 9.0 . . . .

pH approx. saturation concentration [mole/l]	4.0	7.0	9.0
C_o [mole/l] (start conc.)			
C_t [mole/l] (final conc. after t days; t_{max} = 5			
t			
$\dfrac{C_o - C_t}{C_o} \cdot 100\%$ at $50 \pm 0.1°C$			

B. DETERMINATION

- <u>T e s t   s u b s t a n c e</u>

Separate runs at pH 4.0, pH 7.0, and pH 9.0 at the chosen temperature(s) <u>with replication at one of these</u> (the middle temperature in the case of determination at elevated temperatures).

pH 4.0

buffer solution used: . . . .
temperature: . . . .
approximate saturation concentration: . . . .

t [   ]   0

C_t [mole/l]								
log C_t								

pH 7.0

buffer solution used: . . . .
temperature: . . . .
approximate saturation concentration: . . . .

t [   ]   0

C_t [mole/l]								
log C_t								

"Hydrolysis as a Function of pH"

pH 9.0

buffer solution used: ....

temperature: ....

approximate saturation concentration: ....

t [   ] 0

C_t [mole/l]								
log C_t								

• **H y d r o l y s i s   a t   p H   1 - 2**

buffer solution used: ....

pH: ....

temperature: ....

approximate saturation concentration: ....

t [   ] 0

C_t [mole/l]								
log C_t								

• **F i n a l   d a t a**

pH	temperature in °C	start concentration C_o in mole/l	reaction rate constant k_{obs} in l/s x 10^5	half life $t_{1/2}$ in h	coefficient of correlation r^2

C. REPORT ON TEST METHOD

•   <u>Detailed description of the experimental conditions, e.g.</u>

–   for maintaining sterility
–   to avoid photolytic effects
–   to exclude oxygen
–   to prepare the test solution

(Please use another sheet of paper.)

•   <u>Analytical combinations used</u>

pH electrodes	
UV-visible spectrophotometry	
conductivity	
gas chromatography	
high pressure liquid chromatography	
extraction and formation of derivative(s)	

•   <u>Details of the analytical performance</u>

Type of apparatus: ....
Test conditions: ....

•   <u>Do you ascertain the accuracy of this result in any additional way ?</u>

Particular incidents:

Comments:

OECD GUIDELINE FOR TESTING OF CHEMICALS

112

Adopted:
12 May 1981

"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.

---

*Users of this Test Guideline should consult the Preface,
in particular paragraphs 3, 4, 7 and 8.*

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

$$RX \rightleftharpoons R^+ + X^-$$

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

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

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 componds 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)	Temp. in °C
— 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)	Temp. in °C
	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.

- <u>Quality criteria</u>

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.

- <u>Preparations</u>

      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.

- <u>Test conditions</u>

Temperature

      The temperature should be controlled to at least ± 1°C. The determination should preferably be carried out at 20°C.

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 ± 0.1°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 CO_2. 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_o of the salt can be found by extrapolation to zero concentration.