

The Dissociation Constants of Pyruvic and Oxaloacetic Acid

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The two important acids, pyruvic and oxaloacetic acid, are both fairly strong acids, but only very scant information of their dissociation constants is found in the earlier literature¹⁻³. Oxaloacetic acid is unstable in aqueous solution. It decomposes into pyruvic acid and carbon dioxide. In order to give a satisfactory interpretation of the results of a kinetic study of this reaction (preliminary communication⁴), it was desirable to have better data for the dissociation constants of the two acids. They were therefore determined by means of the glass electrode in solutions containing sodium or, in some cases, potassium chloride, at the temperatures 25 and 37° C.

MATERIALS

In the preparation and purification of oxaloacetic acid, the method of Wohl and Oesterlin⁵ was followed with some modifications. First, *diacetyltartaric anhydride* was prepared as described by these authors. Next, the *pyridinium salt of oxymaleic anhydride* was prepared by the following procedure worked out and kindly communicated to me by Dr. A. Klit⁶:

In a 300-ml beaker is placed 80 ml of dry pyridine. At room temperature, and with good stirring by means of a thermometer, 60 g of finely powdered diacetyltartaric anhydride is added. After 15-30 seconds, when practically all has gone into solution with a yellow to green colour, 24 ml of glacial acetic acid is run into the beaker. The turbidity disappears, and the temperature rises to about 40°. The mixture is quickly cooled in a good ice-salt bath while it is stirred vigorously by means of the thermometer. Very soon precipitation of the pyridine compound sets in. Owing to the heat generated by the reaction, the temperature has at first a tendency to rise in spite of good cooling. When it falls, it is best to keep it for about two minutes between 30 and 25° since the reaction stops at about 20°. The mixture is cooled to 0° and 100 ml of dry ether is added. The ether promotes the precipitation of the pyridinium salt. The mixture is then filtered by suction on a glass filter. The salt is washed twice with absolute alcohol and two or three times with dry ether, both liquids being strongly cooled. The ether is removed by sucking air through the preparation. In order to prevent condensation of water from the air, it is

necessary to heat the filter with the crystals. It is therefore placed in a steam-heated drying box for one or two minutes, after which air is again sucked through the crystals. This is repeated until the preparation does not smell of ether. The preparation is only faintly green. It melts at 108–110° C. Yield 42 g or 78 per cent of the theoretical amount based on diacetyltartaric anhydride.

Finally, *oxaloacetic acid* was prepared by the following procedure. 36 g of the pyridinium salt of oxymaleic anhydride was, in small portions, dissolved in 120 ml of 38 per cent hydrochloric acid. During the addition, the solution heated itself a little. When all was dissolved, oxaloacetic acid began to deposit. At the same time, the colour of the solution changed from greenish blue to reddish. The mixture was placed in a good ice-salt bath where it stayed for several hours. It was then filtered by suction, and the crystals were washed twice with altogether 50 ml of concentrated hydrochloric acid, which had first been cooled in an ice-salt bath. They were dried in vacuum over concentrated sulphuric acid and solid sodium hydroxide. The dry preparation had a faint greenish-gray colour. The yield of crude acid was 90 per cent of the theoretical amount.

Recrystallisation of oxaloacetic acid. 25 g of the crude acid was dissolved in 205 ml of hot acetone. 325 ml of 55° warm benzene was added to the nearly boiling solution. On cooling in an ice-salt bath, oxaloacetic acid crystallised. After filtration, it was washed with benzene and dried in vacuum over concentrated sulphuric acid and paraffin-wax shavings. Yield 18 g. More, but less pure, acid could be obtained by partial evaporation of the mother liquor. The recrystallised acid was nearly white with a faint brown tint, which remained unchanged after renewed recrystallisation. It was not hygroscopic and could be stored in an ordinary glass-stoppered vessel without decomposing. The oxaloacetic acid used for the measurements had been recrystallised twice. The molecular weight found by dissolving a sample in a small excess of sodium hydroxide and titrating back with hydrochloric acid, using phenolphthalein as an indicator, was 132.3 (calc. 132.1).

Two preparations of *pyruvic acid* were used for the measurements:

A. Pyruvic acid prepared according to *Organic Syntheses*⁷ and fractionated twice under reduced pressure. The fraction used distilled from 68.9 to 69.4° C at 17 mm Hg. The molecular weight found by alkalimetric titration was 0.2 per cent too low.

B. A Merck preparation which was further purified by two fractional distillations under reduced pressure, followed by two partial crystallisations of the constant-boiling fraction. The final product had the melting point 11° C and a molecular weight found by alkalimetric titration which was 0.6 per cent too high.

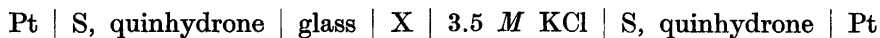
The other chemicals used were of analytical purity. Redistilled water was used to make up the solutions.

EXPERIMENTAL METHOD

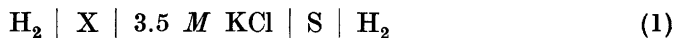
The procedure was essentially the same as that used by the author in earlier papers⁸⁻¹⁰. Glass electrodes with a plane membrane made of Corning glass no. 015 were applied. The electrodes were at room temperature immersed into the solutions to be measured, and the cells were placed in an electrically heated liquid-paraffin thermostat for temperature adjustment. The e.m.f. was measured by means of a valve potentiometer. No hysteresis effect owing to the

cooling of the electrodes to room temperature between the measurements was observed (*cf.* Irving and Williams¹¹). Only such electrodes were used which, with sufficient accuracy, showed the same potential change as the hydrogen electrode when transferred from the solution 0.01 molar HCl, 0.09 molar NaCl to a phosphate buffer of pH 6.81.

The cells examined had the composition



where S denotes the solution 0.00999 *M* HCl, 0.0900 *M* NaCl and X the solution under investigation. When a small asymmetry potential, measured daily by substituting S for X, was subtracted, the e.m.f. *E* volts of cells of the composition



was obtained. The solution X contained oxaloacetic or pyruvic acid (*a* molar), sodium or potassium chloride (*s* molar) and, sometimes, sodium hydroxide (*b* molar). *a* varied from 0.01 to 0.02; in a few cases, it was higher or lower. *s* varied from 0.02 to 0.3. The solutions were made up at room temperature, and no correction was applied for their expansion when they were heated to 25 or 37° C. If a correction were applied, all concentrations and dissociation constants given in this paper for the temperature 37° C should be diminished by 0.5 per cent, while 0.002 should be added to the values of $-\log K$. In the measurements at 25° C, the correction is without any importance.

The hydrogen ion concentration $[\text{H}^+]$ of solution X may be found from the measured values of *E* by means of the following equations when *A* is known:

$$-\log [\text{H}^+] = A + 16.90 E \quad (\text{at } 25^\circ \text{C}) \quad (2)$$

$$-\log [\text{H}^+] = A + 16.25 E \quad (\text{at } 37^\circ \text{C}) \quad (3)$$

A includes the salt effect and the liquid-liquid effect of the left half cell of scheme 1, together with a constant part representing the whole effect of the right half cell. For *A* was used an approximate value determined for a solution which did not contain the organic acid but, instead of the anions of this acid, an equivalent amount of chloride ions. *E* was therefore measured also for solutions X containing only hydrogen chloride (from 0.01 to 0.001 molar) and sodium or potassium chloride (from 0.3 to 0.02 molar). From the known hydrogen ion concentrations in these solutions, the values of *A* given in Table 1 were found by means of equations 2 and 3. When they are used for the solutions containing

Table 1. Values of A in equations 2 and 3 for solutions of the composition: h molar hydrogen chloride, s molar sodium or potassium chloride.

		h	A when $s =$				
			0.30	0.20	0.10	0.05	0.02
NaCl,	25°	0.0100	2.014	2.005	2.000	2.001	2.001
		0.0050	2.015	2.003	2.000	2.002	2.004
		0.0020	2.014	2.004	2.001	2.006	2.014
		0.0010	2.012	2.004	2.003	2.010	2.016
KCl,	25°	0.0100	1.979	1.977	1.983	1.992	1.997
		0.0050	1.978	1.978	1.987	1.992	2.001
		0.0020	1.981	1.981	1.986	1.998	2.009
		0.0010	1.983	1.982	1.987	2.002	2.012
NaCl,	37°	0.0100	2.014	2.006	2.000	2.001	2.000
		0.0080				2.004	2.003
		0.0060		2.007		2.006	2.002
		0.0050	2.016		2.002	2.008	2.008
		0.0020	2.017	2.005	2.003	2.011	2.016
		0.0010	2.015	2.006	2.006	2.012	2.023
KCl,	37°	0.0100	1.978	1.978	1.984	1.992	1.999
		0.0080			1.984	1.994	2.002
		0.0050	1.980	1.980	1.990	1.998	2.008
		0.0020	1.980	1.981	1.989	2.002	2.014
		0.0010	1.983	1.984	1.993	2.001	2.015

the organic acids, they give a better approximation the greater the concentration s of salt added. The error may be divided into two parts. The first part is due to the change of the ideal diffusion potential (Guggenheim¹²), the second to the change of activity coefficients when the organic acid is replaced by hydrochloric acid. The magnitude of the first part may be estimated by means of Henderson's formula^{12,8}. It was found that it, in the most unfavourable cases, corresponds to an error in $-\log [H^+]$ of about 0.002. The magnitude of the second part of the error cannot be estimated beforehand, but its possible influence will be discussed later.

THE DISSOCIATION CONSTANT OF PYRUVIC ACID

The composition of the solutions measured and the results obtained are given in Tables 2 and 3 for the temperatures 25 and 37° C, respectively. The solutions were made from 0.2 molar standard solutions of pyruvic acid (either

Table 2. The dissociation constant K of pyruvic acid at 25° C in the solution: a molar pyruvic acid, b molar sodium hydroxide, s molar sodium chloride. Preparation A of pyruvic acid was used except for the data in the column marked B, which were obtained from measurements with preparation B.

<i>a</i>	<i>b</i>	<i>s</i>	<i>E</i>	$-\log[H^+]$	$-\log K$	$-\log K$ B	$\Delta 10^3$
0.02003	0	0.300	0.00275	2.060	2.174	2.173	+ 22
0.02003	0.00505	0.300	0.01385	2.248	2.189	2.189	+ 6
0.02003	0.00999	0.300	0.0278	2.484	2.191	2.188	+ 6
0.01002	0	0.300	0.01455	2.260	2.176	2.181	+ 18
0.01002	0.00500	0.300	0.0367	2.634	2.200	2.200	- 5
0.02003	0	0.200	0.0040	2.073	2.210		- 2
0.02003	0.00505	0.200	0.0151	2.259	2.212		- 4
0.02003	0.00997	0.200	0.0291	2.497	2.215		- 8
0.01002	0	0.200	0.01575	2.270	2.208		+ 2
0.01002	0.00499	0.200	0.03795	2.645	2.226		- 17
0.02003	0	0.100	0.0050	2.084	2.241		+ 10
0.02003	0.00505	0.100	0.0164	2.277	2.249		+ 1
0.02003	0.00999	0.100	0.0308	2.521	2.254		- 7
0.01002	0	0.100	0.01685	2.285	2.254		- 1
0.01002	0.00500	0.100	0.0391	2.662	2.260		- 9
0.02003	0	0.050	0.00575	2.098	2.277		+ 19
0.02003	0.00505	0.050	0.0173	2.294	2.284		+ 10
0.02003	0.00999	0.050	0.0318	2.541	2.287		+ 4
0.01002	0	0.050	0.0173	2.294	2.281		+ 19
0.01002	0.00500	0.050	0.0399	2.679	2.294		+ 4
0.02003	0	0.020	0.0061	2.104	2.293	2.292	+ 52
0.02003	0.00505	0.020	0.01795	2.307	2.310	2.311	+ 31
0.02003	0.00999	0.020	0.03295	2.566	2.327	2.325	+ 9
0.01002	0	0.020	0.0178	2.305	2.315	2.314	+ 37
0.01002	0.00500	0.020	0.0408	2.702	2.340	2.336	+ 9

preparation A or B), which were standardised against sodium hydroxide solution. The measurements at 25° were mainly carried out with preparation A, those at 37° mainly with preparation B, but at both temperatures some measurements were repeated with solutions of nearly the same composition but made from the other preparation. In the next to the last column of the tables, $-\log K$ calculated from these measurements is given. The agreement is always satisfactory.

Table 3. The dissociation constant K of pyruvic acid at 37°C in the solution: a molar pyruvic acid, b molar sodium hydroxide, s molar sodium chloride. Preparation B of pyruvic acid was used except for the data in the column marked A, which were obtained from measurements with preparation A.

a	b	s	E	$-\log[\text{H}^+]$	$-\log K$	$-\log K$ A	$\Delta 10^3$
0.03974	0	0.300	-0.00995	1.852	2.114		+ 13
0.03974	0.00999	0.300	0.0028	2.060	2.112		+ 15
0.01987	0	0.300	0.00165	2.041	2.114		+ 13
0.01987	0.00505	0.300	0.01265	2.221	2.122		+ 5
0.01987	0.00999	0.300	0.0270	2.456	2.131		- 4
0.00994	0	0.300	0.0142	2.246	2.122		+ 5
0.00994	0.00500	0.300	0.03635	2.608	2.128		- 1
0.01987	0	0.200	0.0025	2.047	2.131	2.129	+ 7
0.01987	0.00505	0.200	0.0138	2.231	2.144	2.143	- 7
0.01987	0.00999	0.200	0.02805	2.463	2.144	2.142	- 7
0.00994	0	0.200	0.01495	2.250	2.135	2.143	- 1
0.00994	0.00500	0.200	0.03745	2.615	2.144	2.142	- 5
0.01987	0	0.100	0.0036	2.058	2.164		+ 14
0.01987	0.00505	0.100	0.0148	2.242	2.169		+ 7
0.01987	0.00999	0.100	0.0295	2.481	2.175		0
0.00994	0	0.100	0.01575	2.258	2.161		+ 19
0.00994	0.00500	0.100	0.0388	2.634	2.186		- 7
0.01987	0	0.050	0.00425	2.072	2.201		+ 22
0.01987	0.00505	0.050	0.01585	2.264	2.215		+ 6
0.01987	0.00999	0.050	0.0309	2.511	2.227		- 10
0.00994	0	0.050	0.01635	2.273	2.209		+ 18
0.00994	0.00500	0.050	0.0397	2.655	2.233		- 8
0.01987	0	0.020	0.0047	2.078	2.217		+ 54
0.01987	0.00505	0.020	0.01655	2.276	2.240		+ 27
0.01987	0.00999	0.020	0.03155	2.525	2.250		+ 11
0.00994	0	0.020	0.01705	2.284	2.244		+ 34
0.00994	0.00500	0.020	0.0403	2.671	2.266		+ 7

In order to analyse the results and make an attempt to extrapolate the values of $-\log K$ to zero ionic strength, $-\log K + 2\alpha\sqrt{I}$, where α is the Debye-Hückel coefficient and I the ionic strength of the solution, was plotted against I (fig. 1). It is seen from the graph that the points corresponding to the two temperatures fit moderately well to the two straight lines drawn when s is 0.1

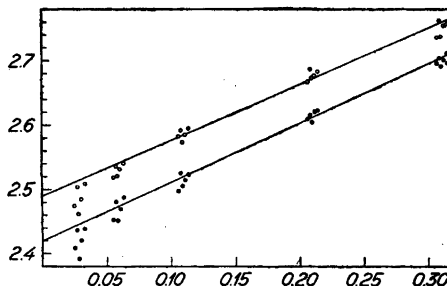


Fig. 1. Abscissa: the ionic strength I , ordinate: $-\log K + 2\alpha\sqrt{I}$, where α is the Debye-Hückel coefficient and K is the dissociation constant of pyruvic acid measured at 25°C (open circles) and at 37°C (solid circles).

or greater. When s is 0.02, the points fall, however, much below the straight lines. On the whole, the points are rather scattered, especially those corresponding to $s = 0.02$. This shows that the activity coefficients of the ions in the solutions are not with sufficient accuracy determined only by the ionic strengths of the solutions, but change perceptibly when a part of the chloride ions are substituted by an equal concentration of pyruvate ions and the concentration of undissociated pyruvic acid is increased. The salt effect error in A mentioned before may therefore have an influence on the values of $[\text{H}^+]$ and K determined. The scattering of the points is consequently explained partly by this error in K , partly by the fact that the true value of K depends not only on the ionic strength but also on the individual nature of the constituents of the solutions. Both effects will decrease when the concentration of sodium chloride increases. We shall therefore assume that the straight lines in fig. 1 represent the true values of K when the concentration of pyruvic acid is small compared with that of sodium chloride, and that their intercepts on the ordinate axis determine, with a fair degree of accuracy, the activity constants K_a at the two temperatures. As a result of these considerations, we obtain the following formulae for the dissociation constant of pyruvic acid:

$$\text{(at } 25^\circ\text{C)} \quad -\log K = 2.490 - 1.013\sqrt{I} + 0.87 I \quad (4)$$

$$\text{(at } 37^\circ\text{C)} \quad -\log K = 2.420 - 1.038\sqrt{I} + 0.92 I \quad (5)$$

It follows from the formulae that K_a is 3.24×10^{-3} at 25°C and 3.80×10^{-3} at 37°C . The difference Δ between $-\log K$ calculated from the formulae and found directly from the measurements is given in the last column of Tables 2 and 3. Böeseken, Hansen, and Bertram³ have determined the dissociation constant of pyruvic acid at 25°C by conductance. They found 3.2×10^{-3} , a value which agrees well with that found in the present paper.

THE FIRST DISSOCIATION CONSTANT OF OXALOACETIC ACID

The solutions were prepared in the following way. The necessary amount of standard sodium or potassium chloride and, in some cases, sodium hydroxide were placed in a 100-ml graduated flask and diluted to about 90 ml. Oxaloacetic acid was weighed out in a small platinum bucket, which was dropped into the solution. When the acid was dissolved, water was added to the mark. Immediately after, the cell was made up and placed in the thermostat. Readings began as soon as temperature equilibrium had been attained. Although decarboxylation takes place with a considerable velocity (half of the acid is decomposed in about 100 minutes at 37°, in nearly 5 hours at 25°), the e.m.f. measured changed only very little with time. It was therefore easy to find the values of E corresponding to undecomposed oxaloacetic acid. They are given in Tables 4 and 5. In Table 5 are also given values of E_{∞} found when the solu-

Table 4. The first dissociation constant K_1 of oxaloacetic acid at 25° C in the solution: a molar oxaloacetic acid, b molar sodium hydroxide, s molar sodium chloride. Data for solutions containing potassium chloride instead of sodium chloride are given in the last column.

a	b	s	E	$-\log[H^+]$	$-\log K_1$	$\Delta 10^3$	$-\log K_1$ (KCl)
0.02004	0	0.300	0.00315	2.067	2.219	+ 5	
0.01503	0	0.300	0.0078	2.146	2.220	+ 3	
0.01000	0	0.300	0.01465	2.263	2.228	- 4	
0.02002	0	0.200	0.00445	2.079	2.249	- 1	2.257
0.02000	0.00719	0.200	0.0202	2.346	2.250	- 2	
0.01500	0	0.200	0.0091	2.158	2.251	- 2	2.266
0.01003	0	0.200	0.0158	2.271	2.252	- 2	2.271
0.02000	0	0.100	0.00595	2.101	2.303	0	2.306
0.02000	0.00721	0.100	0.0223	2.377	2.298	+ 2	
0.01500	0	0.100	0.01055	2.178	2.302	+ 2	2.302
0.01000	0	0.100	0.01725	2.292	2.307	- 2	2.321
0.02000	0	0.050	0.00675	2.115	2.336	+ 18	2.335
0.02000	0.00719	0.050	0.0235	2.400	2.335	+ 15	
0.01501	0	0.050	0.01115	2.190	2.331	+ 25	2.347
0.01002	0	0.050	0.0179	2.305	2.341	+ 17	2.340
0.02000	0	0.020	0.0074	2.127	2.365	+ 43	
0.01502	0	0.020	0.0119	2.204	2.366	+ 45	
0.01002	0	0.020	0.0185	2.318	2.374	+ 40	

tion had been kept at 37° C for one day. At that time, all the oxaloacetic acid had been transformed into pyruvic acid. E_{∞} is a little greater than E , from which it is concluded that the hydrogen ion concentration decreases slightly when the reaction goes on. Pyruvic acid is a little stronger than oxaloacetic acid, for which reason $[H^+]$ should increase, but this effect is more than compensated by a slight second dissociation of oxaloacetic acid, which tends to make $[H^+]$ greater at the beginning of the reaction. The values of E_{∞} found here should correspond to solutions of pure pyruvic acid. They may therefore be compared with E in Table 3 for solutions of the same composition but prepared directly from pyruvic acid. E_{∞} is on an average about 0.0002 volts greater than E in Table 3. The agreement may be considered satisfactory.

In the computation of the first dissociation constant K_1 of oxaloacetic acid from the hydrogen ion concentrations measured, a correction has been made for the concentration of divalent oxaloacetate ions by means of the formulae 10—12 for the second dissociation constant K_2 . The results obtained for solutions containing sodium chloride are given in the first six columns of Tables

Table 5. The first dissociation constant K_1 of oxaloacetic acid at 37° C in the solution: a molar oxaloacetic acid, b molar sodium hydroxide, s molar sodium chloride. Data for solutions containing potassium chloride instead of sodium chloride are given in the last column.

a	s	E	E_{∞}	$-\log[H^+]$	$-\log K_1$	$\Delta 10^3$	$-\log K_1$ (KCl)
0.01999	0.300	0.00115	0.0019	2.033	2.122	+ 17	
0.01500	0.300	0.0063	0.0070	2.117	2.134	+ 5	
0.01001	0.300	0.01365	0.0142	2.237	2.146	- 7	
0.02001	0.200	0.00255	0.0029	2.047	2.161	- 6	2.146
0.01500	0.200	0.0075	0.00775	2.128	2.164	- 8	2.153
0.01003	0.200	0.01435	0.01495	2.240	2.154	+ 2	2.151
0.01999	0.100	0.00365	0.0039	2.059	2.189	+ 12	2.189
0.01499	0.100	0.0085	0.0089	2.139	2.190	+ 12	2.209
0.01000	0.100	0.0157	0.0159	2.257	2.198	+ 6	2.217
0.02001	0.050	0.0044	0.0046	2.075	2.230	+ 19	2.221
0.01500	0.050	0.0092	0.0092	2.154	2.230	+ 21	2.236
0.01000	0.050	0.01635	0.01645	2.273	2.242	+ 12	2.250
0.02000	0.020	0.00485	0.00495	2.082	2.245	+ 54	
0.01502	0.020	0.0098	0.0099	2.162	2.250	+ 53	2.259
0.01001	0.020	0.01705	0.0172	2.284	2.271	+ 36	

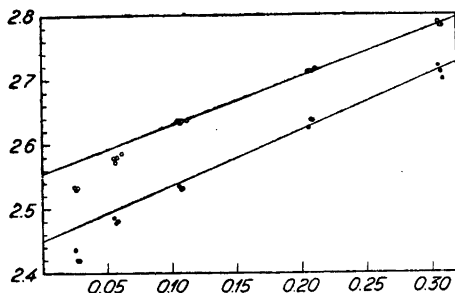


Fig. 2. Abscissa: the ionic strength I , ordinate: $-\log K_1 + 2\alpha\sqrt{I}$, where α is the Debye-Hückel coefficient and K_1 is the first dissociation constant of oxaloacetic acid measured at 25° C (open circles) and at 37° C (solid circles) in solutions containing sodium chloride.

4 and 5. In fig. 2, $-\log K_1 + 2\alpha\sqrt{I}$ has been plotted against I for the same solutions. The two straight lines have been drawn so as to fit best to the points when s is 0.1 or more. When s is lower than 0.1, the points fall below the straight lines. The picture is similar to that found earlier for pyruvic acid, and the discussion given there may also be applied here. If we assume that the straight lines represent the true values of K_1 when the concentration of oxaloacetic acid is small compared with that of sodium chloride, we obtain the following formulae

$$\text{(at 25° C)} \quad -\log K_1 = 2.555 - 1.013 \sqrt{I} + 0.75 I \quad (6)$$

$$\text{(at 37° C)} \quad -\log K_1 = 2.450 - 1.038 \sqrt{I} + 0.86 I \quad (7)$$

It follows from the formulae that the activity constant K_{1a} is 2.79×10^{-3} at 25° C and 3.55×10^{-3} at 37° C. The difference between $-\log K_1$ calculated from the formulae and found directly from the measurements is given in the next to the last column of Tables 4 and 5. In the last column of the tables are given values of $-\log K_1$ found for solutions containing potassium chloride instead of sodium chloride. They do not deviate much from those for sodium chloride, but they are apparently somewhat less regular.

In a preliminary paper⁴ on the kinetics of the decarboxylation of oxaloacetic acid, K_1 was computed exclusively from kinetic data for solutions containing potassium chloride. The following comparison shows that there is a satisfactory agreement between the results obtained by the two methods.

I	0.05	0.10	0.20
$K_1 \times 10^3$ from kinetic data	5.44	6.10	6.75
$K_1 \times 10^3$ from formula 7	5.48	6.21	6.95

No precise measurements of the dissociation constant of oxaloacetic acid are found in the earlier literature. Wohl and Claussner² found by conductance at 17° C for two samples of oxaloacetic acid, considered by them to be respectively oxyfumaric and oxymaleic acid, for K_1 the values 2.76×10^{-3} and 2.50×10^{-3} .

THE SECOND DISSOCIATION CONSTANT OF OXALOACETIC ACID

The solutions were prepared in the same way as those used for the determination of the first dissociation constant but more sodium hydroxide was added (see Table 7). The e.m.f. rose here rapidly with time. It follows from the kinetic study of the reaction¹³ that the concentration of the divalent oxaloacetate ion Ox^{--} remains constant until nearly all the univalent ion HOx^- has disappeared, and that

$$-\frac{d[\text{HOx}^-]}{dt} = k_1[\text{HOx}^-] + k_2[\text{Ox}^{--}]$$

If we divide by $[\text{HOx}^-]$ and introduce decadic logarithms, we obtain

$$-\frac{d\log[\text{HOx}^-]}{dt} = k_1^* + k_2^* \frac{[\text{Ox}^{--}]}{[\text{HOx}^-]} \quad (8)$$

When we use

$$[\text{HOx}^-] = [\text{H}^+] [\text{Ox}^{--}] / K_2$$

and equation 3, we get, for the temperature 37° C,

$$-d\log [\text{HOx}^-] = -d\log [\text{H}^+] = 16.25 dE$$

On introducing this result into equation 8, we find

$$16.25 \frac{dE}{dt} = k_1^* + k_2^* \frac{[\text{Ox}^{--}]}{[\text{HOx}^-]} \quad (9)$$

When the values of E observed for a solution were plotted against t , the points fell close to a straight line for a much greater part of the reaction than we should expect from equation 9. The slope was roughly independent of the

Table 6.

<i>a</i>	<i>b</i>	$\frac{dE}{dt} 10^3$	$\frac{dE}{dt} 10^3$	$-\frac{d\log[H^+]}{dt}$	$\frac{[Ox^{--}]}{[HOx^-]}$	$k_2^* 10^3$
		25°	37°	37°	37°	min ⁻¹
0.200	0.333	0.215	1.035	0.0168	2.09	4.9
0.200	0.300	0.145	0.735	0.0119	1.10	4.9
0.200	0.266	0.107	0.553	0.0090	0.61	4.1

concentration of sodium or potassium chloride added. Average values of the slopes for all the solutions listed in Table 7 are given in Table 6. For the temperature 37° C, an estimate has been made of k_2^* by means of equation 9. For this purpose we have used the ratios of the initial concentrations of the ions $[Ox^{--}]$ and $[HOx^-]$ given in the table and $k_1^* = 6.5 \times 10^{-3} \text{ min}^{-1}$ found earlier⁴. The values computed for k_2^* vary from 4.1 to $4.9 \times 10^{-3} \text{ min}^{-1}$, while we from direct kinetic measurements¹³ should expect about $2 \times 10^{-3} \text{ min}^{-1}$ for the uncatalysed decarboxylation of the divalent ion. The reaction is, however, catalysed by traces of certain metal ions, and it is not excluded that impurities of such ions have been present in the sodium hydroxide used.

In order to find the value E would have if no decomposition of the oxaloacetic acid had taken place, a suitable extrapolation was carried out by means of the straight line in the plot of E against t . The solutions were prepared at room temperature (about 21° C), and 3.5 to 4 minutes elapsed from the dissolution of the acid until the cells were placed in the thermostat. From a previous determination of the temperature-time curve for the cell in the thermostat and knowledge of the variation of $\frac{dE}{dt}$ with temperature, it was found that, in measurements at 37° C, extrapolation should be made to a time 1.5 minutes after, in those at 25° C, to a time 1.5 minutes before the cells were placed in the thermostat. The values of E given in Table 7 have been found by extrapolation in this way. In calculating $-\log [H^+]$ from E , equations 2 and 3 were used together with the values of A corresponding to $h = 0.01$ in Table 1. In the computation of the second dissociation constant K_2 of oxaloacetic acid from the hydrogen ion concentrations measured, a correction was made for the concentration of undissociated acid by means of the values of K_1 measured before. The ionic strengths of the solutions and the values of $-\log K_2$ found by this computation are given in the 6th and 7th column of Table 7. All the

Table 7. The second dissociation constant K_2 of oxaloacetic acid at 25 and 37° C in the solution: a molar oxaloacetic acid, b molar sodium hydroxide, s molar sodium chloride. In the last two measurements (marked *), the solution contained potassium chloride instead of sodium chloride.

a	b	s	E	$-\log[H^+]$	I	$-\log K_2$	$-\log K_2$ calc.
<i>At the temperature 25° C:</i>							
0.02000	0.03328	0.200	0.1219	4.066	0.2468	3.746	3.750
0.02000	0.03004	0.200	0.1063	3.801	0.2406	3.750	3.754
0.02000	0.02659	0.200	0.09175	3.556	0.2343	3.763	3.757
0.02000	0.03328	0.100	0.12665	4.142	0.1468	3.824	3.824
0.02000	0.03004	0.100	0.11095	3.877	0.1406	3.831	3.831
0.02000	0.02659	0.100	0.0961	3.626	0.1342	3.840	3.839
0.02000	0.03328	0.050	0.13085	4.216	0.0968	3.901	3.891
0.02000	0.03004	0.050	0.1145	3.940	0.0905	3.898	3.903
0.02000	0.02659	0.050	0.0993	3.683	0.0841	3.904	3.914
0.02000	0.03004	0.000	0.12135	4.066	0.0404	4.031	4.026
0.01000	0.01502	0.015	0.12085	4.057	0.0353	4.013	4.044
<i>At the temperature 37° C:</i>							
0.01999	0.03003	0.250	0.10705	3.752	0.2906	3.703	3.708
0.02001	0.02660	0.250	0.09155	3.500	0.2843	3.710	3.710
0.02001	0.03331	0.200	0.12505	4.040	0.2469	3.720	3.725
0.02000	0.03003	0.200	0.10875	3.775	0.2406	3.728	3.728
0.02002	0.02662	0.200	0.09365	3.529	0.2343	3.743	3.731
0.02003	0.03004	0.100	0.1132	3.844	0.1405	3.805	3.807
0.01999	0.02658	0.100	0.0982	3.601	0.1341	3.824	3.815
0.02000	0.03004	0.050	0.1172	3.912	0.0905	3.873	3.881
0.02001	0.02664	0.050	0.10175	3.661	0.0841	3.888	3.892
0.02001	0.03003	0.020	0.12115	3.981	0.0604	3.948	3.946
0.02000	0.03010	0.000	0.1245	4.041	0.0405	4.004	4.006
0.01999	0.03007	0.250 *	0.10985	3.771	0.2907	3.720	3.720
0.02001	0.02664	0.250 *	0.0937	3.509	0.2844	3.718	3.721

values agree well with the following formulæ where the square-root term has been calculated according to Debye and Hückel.

$$\text{(at 25° C, NaCl)} \quad -\log K_2 = 4.370 - 2.026\sqrt{I} + 1.57 I \quad (10)$$

$$\text{(at 37° C, NaCl)} \quad -\log K_2 = 4.359 - 2.076\sqrt{I} + 1.61 I \quad (11)$$

$$\text{(at 37° C, KCl)} \quad -\log K_2 = 4.359 - 2.076\sqrt{I} + 1.65 I \quad (12)$$

In the last column of Table 7 are given the values of $-\log K_2$ calculated by means of these formulae. It follows from the formulae that the activity constant K_{2a} is 4.27×10^{-5} at 25°C and 4.38×10^{-5} at 37°C .

SUMMARY

A description is given of a method for the determination of concentration dissociation constants of weak and moderately strong acids in salt solutions by means of electrometric measurements of cells with liquid junction. The limited usefulness of the method is discussed.

The method was used for the determination of the dissociation constant of pyruvic acid and the first and second dissociation constants of oxaloacetic acid in sodium and, in some cases, potassium chloride solutions at the temperatures 25 and 37°C . The results for pyruvic acid are summarised in the formulae 4–5, those for oxaloacetic acid in the formulae 6–7 and 10–12.

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