XI. THE DIALURIC ACID-ALLOXAN EQUILIBRIUM.

By GEORGE MAXWELL RICHARDSON (*1851 Exhibition Research Scholar*) AND ROBERT KEITH CANNAN.

*From the Department of Physiology and Biochemistry,
University College, London.*

(Received January 2nd, 1929.)

IN acid solution, dialuric acid and alloxan may readily be converted into one another. The change is one involving two equivalents of hydrogen:

The addition of one equivalent of a reducing agent to alloxan or of one equivalent of an oxidising agent to dialuric acid leads, on the other hand, to the separation of the sparingly soluble substance, alloxantin. The latter is also formed when solutions of these two reactants are mixed in equivalent proportions, and it has therefore been natural to regard it as a molecular compound comparable to the quinhydrones and meriquinones. It is generally believed that alloxantin is extensively dissociated into its two components in aqueous solution.

Our interest in the above system originated in the demonstration by Biilmann and Lund [1923] that solutions of alloxantin developed constant and reproducible potentials at a platinum electrode in the absence of oxygen. Their observations, which were restricted to solutions of alloxantin in 0.1 and 0.02 *M* sulphuric acid, indicated that the observed potentials (E_h) were related to the hydrogen ion activity by the equation¹

$$E_h = E_0 - \frac{RT}{F} \ln [H^+],$$

E_0 being a constant with a value of 0.3698 at 18° and of 0.3664 at 25°. Assuming that alloxantin dissociated into equivalent amounts of dialuric acid and alloxan, Biilmann and Lund plausibly attributed the potentials of solutions of alloxantin to the reversible oxidation-reduction system

The studies which will be reported in this paper have had as their object the quantitative proof of this assumption, the extension of the electrode data

¹ $[H^+]$ refers throughout to the hydrogen ion activity measured electrometrically. $[a]$, $[b]$ and $[m]$ of the electrode equations are actually molar concentrations, the assumption being made that these are sensibly equal to the activities at the experimental concentrations employed.

to cover the whole significant p_H range, and the examination of the effects upon the electrode behaviour of the system of the formation of alloxantin. It early became apparent that the system was further complicated by the fact that the oxidant was subject to an irreversible change at a rate which was a function of the hydroxyl ion concentration, and it therefore became necessary to relate the kinetics of this change to the electromotive behaviour of the system.

EXPERIMENTAL.

The dialuric acid, alloxan and alloxantin which were employed were prepared from uric acid in the usual way, and their purity established by determinations of nitrogen, base-binding capacity, and (in the cases of dialuric acid and of alloxantin) their iodine-reducing capacities.

In order to comprehend the relations of the equilibrium potentials to $[H^+]$, it was desirable to have independent determinations of those ionisation constants of the reactants which were effective within the experimental p_H range. The acidic constant of dialuric acid was measured with precision by means of a hydrogen electrode titration curve in the usual manner. This titration betrayed no other groups having constants of significant values. The case of alloxan was more difficult. Its ease of hydrogenation renders the hydrogen electrode method inadmissible. Moreover, the degree of ionisation of alloxan determined experimentally appears to change with time. Woods [1906] found that the conductivity of solutions of alloxan increased steadily, and Biilmann and Bentzon [1918] observed that there is also an increase in their $[H^+]$ as determined by colorimetric methods. These effects are, most probably, due to the isomeric change to alloxanic acid which readily takes place under the influence of hydroxyl ions. In an attempt to construct a titration curve by means of colorimetric p_H determinations, we observed this change in p_H in all our mixtures of alloxan and sodium hydroxide. For any given mixture, however, $d(p_H)$ is approximately a linear function of the time. It was, therefore, possible to extrapolate to zero time the p_H values of such mixtures (where α varied from 0.09 to 0.58). The section of the titration curve thus constructed led to a quite consistent series of values for the first (acidic) constant of alloxan. It will be shown, later, that the electrode data indicate the presence of a second acidic constant of significant magnitude as well as a very weak basic group. The constant of the latter seems to be too small for direct measurement [cf. Biilmann and Bentzon, 1918] while the demonstration of the former was not attempted because of the instability of alkaline solutions.

The technique employed in the determination of the electrode potentials has already been outlined [Cannan, 1926]. Both dialuric acid and alloxantin proved to be very prone to autoxidation. It was necessary, therefore, to employ all the usual precautions for the total exclusion of oxygen during the preparation and manipulation of solutions of the reactants. Electrode potential titration curves for the titration of dialuric acid (0.002 M) in 0.05 M buffer

solutions [Clark, 1928] with 0.02 *M* potassium ferricyanide or iodine were obtained at several p_H values between 1 and 5. Both platinum and gold-plated electrodes were employed and generally gave concordant readings. The former, however, were frequently sluggish in reaction, whereas the gold-plated electrodes consistently adjusted themselves to changes in the system within two or three minutes. The main part of the curves traced by titrating with iodine and with ferricyanide were identical, but iodine sometimes gave distortions of the initial and terminal portions of the curves, so rendering less precise the determination of end-points. For the detailed analysis of the data, therefore, we have restricted ourselves to the ferricyanide titrations and the gold-plated electrodes. All electrometric observations were made on solutions maintained at 30° in a thermostat.

The titration data for the relation of the electrode potentials to $[H^+]$ were further elaborated by observations of the equilibrium potentials of 0.001 *M* solutions of alloxantin in a series of 0.05 *M* buffer solutions with p_H values intervening between those of the complete titration curves.

On the alkaline side of p_H 5 steady potentials were never attained, either in solutions of alloxantin or in mixtures of alloxan and dialuric acid. Instead, linear drifts towards increasing negative values were consistently observed. It was, therefore, not possible to conduct titrations in this range, but it proved to be possible to obtain consistent values for the equilibrium potentials of alloxantin by extrapolating to zero time the potential-time drifts. Theoretical justification for this procedure will be given later.

The p_H values of all systems studied were determined directly by means of the hydrogen electrode.

RESULTS.

In Table I are assembled the data for a typical titration curve at p_H 1. At constant p_H the electrode equation for the system dialuric acid-alloxan is

$$E_h = E_0' - 0.03006 \log \frac{[b]}{[a]}, \quad \dots\dots(1)$$

E_0' being a constant, and $[b]$ and $[a]$ being the respective concentrations of dialuric acid and alloxan. The constancy of E_0' when this equation is applied to the data is satisfactory evidence that the potentials are related to a simple reversible system involving two equivalents of hydrogen.

The first three values for E_0' in Table II have been derived from complete titrations in this way. The remaining values are equilibrium potentials of solutions of alloxantin derived by direct observation or by extrapolation. All these and others are displayed in Fig. 1, which sufficiently demonstrates that the alloxantin potentials agree well with the values of E_0' obtained by titration, and that these are plausibly extended into the alkaline ranges by the values obtained by extrapolation of the time-potential drifts. In other words, the potentials of alloxantin may properly be attributed to the presence in solution of equivalent amounts of dialuric acid and alloxan.

Table I.

Titration of 50 cc. of 0.00175 *M* dialuric acid with 0.02024 *M* potassium ferricyanide in 0.1 *M* HCl. Temperature 30°. p_H 1.043.

Titre	$0.03006 \log \frac{[b]}{[a]}$	E_h	E_o'
0.15 cc.	+0.0525	+0.2505	[+0.3030] 0.3044 0.3032 0.3019
0.23	0.0468	0.2576	
0.35	0.0412	0.2620	
0.55	0.0350	0.2669	
0.85	0.0288	0.2730	0.3018
1.15	0.0243	0.2775	0.3018
1.65	0.0187	0.2833	0.3020
2.35	0.0127	0.2888	0.3015
3.15	0.0070	0.2948	0.3018
4.15	+0.0008	0.3011	0.3019
5.15	-0.0054	0.3070	0.3016
6.15	0.0123	0.3138	0.3015
6.95	0.0192	0.3211	0.3019
7.65	0.0279	0.3297	0.3018
8.15	0.0393	0.3415	0.3022
8.35	0.0487	0.3503	0.3016
8.55 (end-point)			Mean 0.30185 $\pm$ 0.00035

Table II.

Relation of E_o' to p_H . $E_o = +0.3640$.

p_H	E_o'		Difference	Method
	Found	Calculated		
1.043	+0.3018	+0.3015	0.0003	Titration of 0.002 <i>M</i> dialuric acid
2.943	0.1976	0.1979	0.0003	
4.884	0.1320	0.1322	0.0002	
2.127	0.2388	0.2385	0.0003	Equilibrium potentials of 0.001 <i>M</i> alloxantin
2.549	0.2143	0.2164	0.0021	
3.153	0.1879	0.1891	0.0012	
3.757	0.1668	0.1675	0.0007	
4.155	0.1549	0.1546	0.0003	
4.565	0.1426	0.1420	0.0006	
4.965	0.1294	0.1297	0.0003	
5.420	0.1155	0.1158	0.0003	
5.885	0.1009	0.1014	0.0005	Extrapolated potentials for 0.001 <i>M</i> alloxantin
6.555	0.0789	0.0790	0.0001	
7.027	0.0602	0.0611	0.0009	
7.494	0.036	0.039	0.003	
8.025	+0.011	+0.011	—	
9.007	-0.052	-0.048	0.004	
9.955	0.112	0.112	—	
10.370	0.146	0.144	0.002	
11.055	0.205	0.202	0.003	
11.955	0.274	0.282	0.008	

Table III.

Dissociation constants (uncorrected for activity). Temperature 30°.

	Group	K'	pK'	Method
Dialuric acid	1st acidic (K_1')	1.48×10^{-8}	2.83	Hydrogen electrode
	2nd "	Negligible	> 11	E_o' : p_H relation
	3rd "	"	> 11	"
Alloxan	1st acidic (K_1')	6.31×10^{-8}	7.20	Colorimetric
	2nd " (K_2')	1.0×10^{-10}	10.0	E_o' : p_H relation
	Basic	Negligible	< 1	"

Fig. 1 betrays a system more complicated than was expected. The "0.09 slope" exhibited at extreme alkalinity together with the fact that both dialuric acid and alloxan have acid properties suggest that the type of system which best fits the structural relations of the reactants is

The values to be assigned to the dissociation constants of the various ionisable hydrogen atoms are given in Table III. The strong acid group of dialuric acid must be assigned to the carbon atom carrying the hydroxyl group, and, according to the scheme, this atom must, therefore, assume basic properties on oxidation. Indeed, alloxan may, as has been suggested by Richter [1911], be

Fig. 1. E'_0 : p_H curve

an oxonium base, though our allocation of constants would give it only negligible basic properties. The remaining acidic hydrogen atoms of the two reactants may, plausibly, be identified with the hydrogen atoms attached to nitrogen:

With the assumptions of Table III, the rational equation relating E'_0 to p_H may be simplified to

$$E'_0 = E_0 + 0.03006 \log \frac{[\text{H}^+]^3 [\text{K}'_1 + \text{H}^+]}{[\text{K}'_1 + \text{H}^+] [\text{K}'_2 + \text{H}^+]}. \quad \text{.....(2)}$$

Adopting a value of +0.3640 for the constant E_0 there may be calculated the theoretical values of E'_0 found in Table II. In acid solutions, where the

experimental values are true equilibrium potentials, the agreement is within the experimental error. In neutral and alkaline solutions, where the quoted values are extrapolated from the time course of a drifting potential, greater latitude must be allowed. When it is pointed out that the significant range of potential drift is less than 0.05 v. and that the observed rates rose to values as great as 0.02 v. per minute, it is apparent that precise extrapolation is quite impossible.

It is well known that the initial equilibrium of an electrode with an active system is frequently much slower than subsequent adjustments to changes in that system. This was so in the present case. The observations on solutions of alloxantin in which the potential drift was rapid were, therefore, made by adding 4 cc. of 0.01 *M* alloxantin to 30 cc. of a deaerated buffer of acid p_H , and then, after about fifteen minutes, adding the necessary amount (about 10 cc.) of sodium hydroxide to bring the solution to the desired p_H . The exact p_H value of the solution was determined by the hydrogen electrode at the conclusion of the experiment. The drift of potential was measured from the time of addition of alkali. Using this method, it was possible by observations every half minute to establish, fairly definitely, the linear drift even in the cases where the change was most rapid. There can be little doubt that the data for the more alkaline solutions do establish—in spite of their crudeness—the reality of the two inflexions in the $E_0' : p_H$ curve at p_H 7.2 and 10 respectively (Fig. 1).

The value we have found for E_0 at 30° is quite consistent with those obtained by Biilmann and Lund at 25° and at 18°. The three values may be accurately combined in the expression

$$E_0 = 0.3784 - 0.00048 t.$$

The degree of dissociation of alloxantin.

In Table I the tacit assumption was made that the active mass of dialuric acid at each stage in the titration was equal to the fraction of the initial concentration which remained unoxidised. The active mass of alloxan was, likewise, identified with the fraction of the initial concentration of dialuric acid which had suffered oxidation. If, however, significant amounts of the two reactants associate to form alloxantin then these assumptions become unsound. Under such conditions it may be shown that the correct equation for a titration at constant p_H is

$$E_h = E_0' - \frac{RT}{2F} \ln \frac{[S_r] - [m]}{[S_o] - [m]}, \quad \dots\dots(3)$$

where $[S_r]$ and $[S_o]$ are the concentrations of total reductant and oxidant respectively, and $[m]$ is the concentration of alloxantin. The latter is defined by

$$K = \frac{[m]}{[a][b]}. \quad \dots\dots(4)$$

Now K has been determined by Biilmann and Bentzon from studies upon the solubility of alloxantin in the presence of varying concentrations of added

alloxan. They arrived at a mean value of 39. This would indicate that the degree of association in 0.001 *M* solution would not be sufficient to give values of $[m]$ significant for equation (3). That we were able to ignore this quantity in Table I agrees with this conclusion. At higher concentrations of reactants, however, the degree of association should lead to a distinct distortion of the normal curve. This we found to be the case in the titration of 0.01 *M* dialuric acid at p_H 1 (Table IV). From the titres may be calculated $[S_r]$ and $[S_0]$ in the usual way and thence equation (3) may be solved for $[m]$. Finally K may be calculated from (4). In view of the fact that the method is not well adapted to the accurate measurement of such an association constant, the values derived in Table IV must be held to be in satisfactory agreement.

Table IV.

Titration of 0.00897 *M* dialuric acid with 0.0508 *M* potassium ferricyanide
 $p_H = 1.080$. $E_0' = +0.2990$.

Titre	E_h	$S_0 \times 10^6$	$S_r \times 10^6$	$m \times 10^6$	K
0.41	+0.2572	413.5	8483	71.8	25
0.71	0.2655	711.6	8128	94.5	19
1.01	0.2709	1006	7789	114.7	17
1.51	0.2773	1490	7214	150.0	16
2.21	0.2836	2151	6434	249.5	21
3.01	0.2899	2886	5569	223.3	16
4.01	0.2964	3773	4527	345.5	24
5.01	0.3030	4629	3520	432.0	32
5.51	0.3061	5046	3031	243.2	18
6.01	0.3095	5454	2549	199.2	16
6.81	0.3159	6091	1799	179.0	19
7.51	0.3231	6633	1162	136.6	21
8.01	0.3306	7016	712	97.0	23
8.31	0.3375	7241	445	69.7	26
8.82 (end-point)					Mean 21

Table V.

Velocity of decomposition of alloxan. Temperature 30°.

p_H	Change of E_h per min.	$k = \frac{1}{t} \ln \frac{a}{a-x}$
4.97	-0.000034	0.0026
5.31	-0.00010	0.0077
5.74	-0.00016	0.012
6.00	-0.00037	0.028
6.01	-0.00080	0.061
6.56	-0.0027	0.21
7.03	-0.012	0.92
7.49	-0.018	1.4
7.99	-0.020	1.5
8.03	-0.020	1.5
8.80	-0.016	1.2
9.00	-0.020	1.5
9.01	-0.020	1.5
9.79	-0.016	1.2
9.96	-0.014	1.1
10.37	-0.018	1.4
11.06	-0.016	1.2

Our mean value will be seen to be about half that of Biilmann and Bentzon. Since the p_H of the saturated solutions with which these authors worked would be about 2.4, the discrepancy is much too small to be due to a primary

effect of hydrogen ion activity. More plausibly it may be attributed to the difference in temperature, and in ionic strength, of the solutions in the two studies. We may conclude as a first approximation that K is independent of $[H^+]$ in this range.

The rate of isomeric change of alloxan.

There is little doubt that the drifts of potential of solutions of alloxantin towards more negative values are due to a slow loss of active oxidant. Since they are accelerated by hydroxyl ions and parallel the increases in acidity and conductivity of solutions of alloxan, it is most probable that they are due to the isomeric change of the latter to alloxanic acid. Assuming, therefore, the change to be monomolecular, we have for a buffered solution of alloxantin

$$kt = \ln \frac{a}{a-x},$$

where a is the initial concentration of alloxan and $a - x$ the concentration at time t . At this time, also,

$$E_0' - E_h = \frac{RT}{2F} \ln \frac{a}{a-x}.$$

Hence

$$E_0' - E_h = \frac{RT}{2F} kt. \quad \dots\dots(5)$$

Fig. 2.

A. Isomerism of alloxan (monomol. velocity constant).

B. % autoxidation of dialuric acid per min.

The increase in negative potential should, therefore, be a linear function of the time. As has been already indicated, this agrees with observation. Equation (5) has been employed for the calculation of k from the electrode data at various $[H^+]$. In solutions of p_H greater than 7 the half-life period of alloxan at 30° appears to be only about one minute. The determination of k can, therefore, be only approximate. Nevertheless, as Table V and Fig. 2 indicate,

the velocity appears to be a function of the first acidic constant of alloxan. The curve in the figure represents the theoretical relation of $\log k$ to p_H were the velocity a function of the degree of dissociation of alloxan. Beyond p_H 10—coinciding with the intervention of the second (hypothetical) acidic constant—the potential drift falls away from a linear relation although the initial velocities remain of the same order of magnitude.

It may be added that the rates of change in p_H of mixtures of alloxan and sodium hydroxide give general confirmation of the velocities of change indicated above.

The autoxidation of dialuric acid.

To assist in defining the precautions necessary for the electrode studies, a few observations were made upon the rates of autoxidation of dialuric acid in buffered solutions. Between p_H 5 and 2 the rates were very great but fell away rapidly below this.

It was found that when the reaction was conducted by blowing oxygen through a solution of dialuric acid the velocity was greatly affected by the degree of agitation. Consequently, the following method was adopted. Solutions of 0.004 *M* dialuric acid (oxygen-free) were mixed in a closed chamber with five volumes of oxygenated buffer at zero time. Samples were removed at intervals, added to sufficient hydrochloric acid to depress autoxidation, and titrated in the absence of oxygen with 0.005 *M* iodine.

The rate of autoxidation was found to be roughly linear, with a tendency to fall away in the later stages. This was probably due to a significant diminution of the partial pressure of oxygen. The results were not closely reproducible, there being, apparently, uncontrolled catalytic effects. The latter did not however appear to be of the heavy metal type, since the addition of hydrocyanic acid was without notable effect. In Fig. 2 are plotted logarithms of the rates (percentage oxidation per minute) at various p_H values. The comparison curve gives the relation which would be expected were the velocity a direct function of the degree of dissociation of the dialuric acid.

SUMMARY.

1. The equilibrium potentials of the reversible oxidation-reduction system dialuric acid-alloxan have been determined for the p_H range 1–6 and related to the equilibrium potentials of alloxantin observed by Biilmann and Lund.
2. The association constant of alloxantin has been derived from the electrode data.
3. Equilibrium potentials are not obtained in solutions of p_H above 6. This is probably due to the slow isomeric change of alloxan to alloxanic acid. Upon this assumption it has been possible to derive approximate equilibrium potentials for the range p_H 6–12.

4. The first acidic constants of dialuric acid and of alloxan have been determined. A second acidic constant of alloxan is inferred from the electrode behaviour of the system and a value for it derived.

5. Observations have been made on the relation to p_H of the rates of isomeric change of alloxan and of autoxidation of dialuric acid.

One of us (R. K. C.) is indebted to the Medical Research Council for a grant from which the expenses of this work have been defrayed.

REFERENCES.

- Biilmann and Bentzon (1918). *Ber. deutsch. chem. Ges.* **51**, 522.
Biilmann and Lund (1923). *Ann. Chim.* [9] **19**, 137.
Cannan (1926). *Biochem. J.* **20**, 927.
Clark (1928). The determination of hydrogen ions (Baltimore).
Richter (1911). *Ber. deutsch. chem. Ges.* **44**, 2155.
Woods (1906). *J. Chem. Soc.* **89**, 1831.