

## 7. *A Polarographic Study of the Electroreduction of Acridine.*

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The reduction of acridine at the dropping-mercury electrode has been studied over the pH range 0—14. In aqueous solution, complex anomalous polarograms are obtained. In solutions containing upwards of 50% alcohol, the anomalies disappear, leaving two one-electron reduction steps which are thermodynamically irreversible, and for which diffusion currents are proportional to acridine concentrations in the range  $10^{-3}$ — $10^{-4}$ M. The anomalies in aqueous solutions may be satisfactorily explained on Brdička's theory as due to adsorption of acridine, mainly in the semiquinone reduced state, on the mercury electrode. Supporting evidence for adsorption has been obtained from a cathode-ray oscillographic study of the current-time relation during the formation of a mercury drop at the electrode. Acridine is found to undergo a two-step electroreduction, both in aqueous and alcoholic solutions, with the formation of an intermediate semiquinone radical, the apparently great stability of which is attributed to resonance.

THE behaviour of acridine and some of its derivatives at the dropping-mercury electrode has been studied by Breyer, Buchanan, and Duewell (*J.*, 1944, 360), who concluded that in general these compounds are reduced in two one-electron stages, with the intermediate formation of a semiquinone radical. However, certain anomalies appear in their results. For instance, they describe a three-stage reduction of acridine as well as of 4-aminoacridine; for acridine, the first reduction wave has a constant height for concentrations between  $10^{-4}$ M. and  $10^{-3}$ M., from which they deduce that acridine exists only as a dimer at concentrations exceeding  $10^{-4}$ M. This deduction seems doubtful, since, even if dimerisation did occur, the mass-action law would indicate some increase of wave height with rising concentration. It may be noted that the above workers reported a shift of the half-wave potential of this first reduction step with varying acridine concentration.

These anomalies, and the aim of correlating redox properties of the acridines with their biological activity, have prompted the present investigation.

### EXPERIMENTAL.

Acridine was purified by repeated crystallisation from aqueous alcohol, conversion into hydrochloride, recrystallisation from water, drying until water of crystallisation was removed, reconversion into free base, and again crystallisation from aqueous alcohol. The m. p. of the product was  $107^{\circ}$  (lit.,  $107^{\circ}$ ,  $110^{\circ}$ ,  $111^{\circ}$ ). Mercury for the dropping electrode was purified by treatment with potassium permanganate followed by dilute nitric acid, and then vacuum-distilled.

A manually operated polarographic circuit was used. The potential was applied to the cell by means of a potential divider giving steps of 10 mv., and the cell current was measured by a suitably damped Cambridge "Spot" galvanometer calibrated by measuring the voltage drop across a standard resistance in series; this calibration was frequently checked, but no variation was observed. The polarographic cell (Fig. 1), constructed from a B40 Pyrex glass joint, was similar to one described by Kolthoff and Lingane ("Polarography," Interscience Publishers Inc., N.Y., 1946, p. 245). It contained an immersion-type saturated calomel electrode, against which the potential of the dropping-mercury electrode was measured potentiometrically. The hand-drawn Pyrex capillary had the following characteristics: drop time  $t = 4.2$  secs. with no applied potential; mercury flow rate  $m = 0.902$  mg./sec. in 50% alcoholic buffer at pH 8.29 (measured in the alcoholic solution).

Solutions in the cell were deoxygenated, before being polarographed, by bubbling through them for 20—30 minutes cylinder nitrogen, purified by passage through a series of wash-bottles containing sodium dithionite solution with a little indigo-carmin redox indicator, to give warning of approaching exhaustion of the dithionite.

Cell solutions were made by dissolving known weights of acridine in known volumes of aqueous supporting electrolyte and diluting them with more of the latter as required; when alcoholic solutions were used, the acridine was dissolved in a suitable volume of absolute alcohol (previously shown to contain no electro-active matter), which was then diluted with aqueous buffer and adjusted to a suitable final volume after mixing and cooling to  $25^{\circ}$ .

As supporting electrolytes, the following Sørensen buffer solutions, prepared according to Clark ("The Determination of Hydrogen Ions," 1928) were used: pH 1.925 and 3.95, citrate-hydrochloric acid; pH 5.58 and 7.38, phosphate mixtures; pH 9.07, borate-hydrochloric acid; pH 11.0, borate-sodium hydroxide. Some determinations were made with supporting electrolytes containing various percentages of alcohol. For these solutions, the apparent pH values, as determined by a glass-electrode pH meter and checked with a hydrogen electrode, were somewhat higher than those obtained by indicators (cf.

Shreve and Markham, *J. Amer. Chem. Soc.*, 1949, **71**, 2993); the latter values did not differ significantly from the values for the given buffers in purely aqueous solution. All measurements were at 25°, unless otherwise indicated.

To determine the current-time relationships during the life-time of a single drop at the dropping-mercury electrode, an "Ultrascope" Mark I oscilloscope was employed. The polarographic current (max. value about 3  $\mu$ a.) was amplified by a circuit (Fig. 2) using high-gain valves  $V_1$  and  $V_2$ , which both have +60 v. applied to their screening grids, and small capacities interposed between grid and earth and anode and earth to guard against possible H.F. oscillations in the amplifier.  $B_1$  and  $B_2$  are standard 120-v. high-tension batteries, and potential divider  $R_3$ - $R_4$  is connected across a 48-v. section of  $B_1$ . Linear amplification up to about 4  $\mu$ a. input is obtained by adjusting  $R_3$  until the anode current of  $V_2$  is 0.7 ma., thus achieving the critical setting of the  $V_2$  grid potential necessary to work  $V_2$  near the midpoint of its characteristic curve. Changes in dropping-mercury electrode potential affect the plate current drawn by  $V_1$ , thus necessitating resetting the  $V_2$  grid potential by readjusting  $R_3$  and perhaps also the tapping from  $R_4$  on  $B_1$ . The amplifier output was fed to the Y plates of the oscilloscope,

FIG. 1.

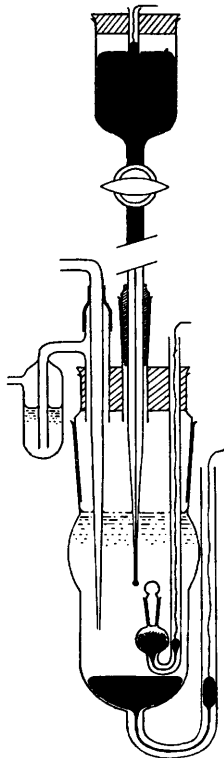
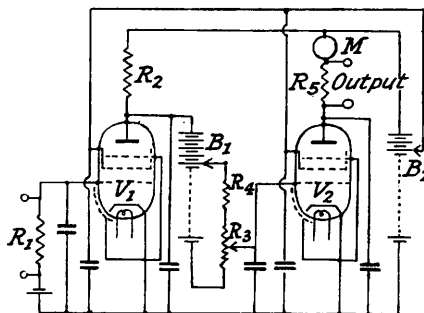


FIG. 2.



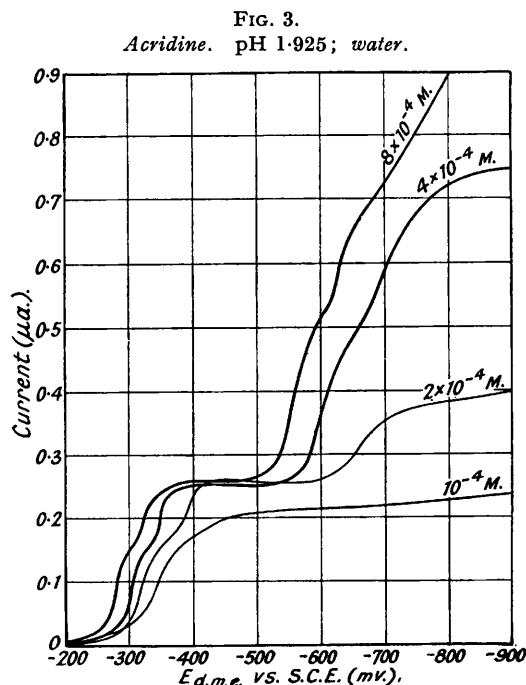
$R_1, R_3, R_4 = 10,000$  ohms.  
 $R_2 = 50,000$  ohms.  
 $R_5 = 100,000$  ohms.  
 Capacities of all condensers = 0.001  $\mu$ F.  
 $V_1, V_2 = \text{EF.50 (Mullard)}$ .

whilst to the X plates was connected a 2  $\mu$ F. condenser which, charged by a 400-v. supply through a 1-megohm series resistance, constituted a simple time base; this, on closing of the circuit, gave a sweep which, although linear over the early portion only, was adequate for the immediate purpose. To photograph an oscillogram, the potential of the dropping-mercury electrode was set at the desired value, the grid potential of  $V_2$  adjusted as described above, and the oscilloscope was then adjusted so that the spot was almost off the left-hand edge of the 6-in. diameter screen, where its periodic vertical deflections, due to the growth and fall of the mercury drops, could still be followed. The shutter of a camera, focused on the screen, was then opened, and the time-base switch closed so that the fall of the mercury drop occurred after the spot had travelled about 1 in. across the screen, *i.e.*, about  $\frac{1}{4}$  second after closing the switch; after the spot had completely traversed the screen, the shutter was closed. The delicate timing of the operation was aided by listening to a pocket watch giving 5 ticks per second. Difficulties due to pick-up of 50-cycle mains A.C. ripple in the leads to the amplifier were minimised by screening the leads and earthing the amplifier and oscilloscope. A slight 50-cycle ripple still remained in the oscillogram, but this was deliberately retained, since it provided a useful automatically recorded time scale. The amplifier-oscilloscope combination was simultaneously checked for linearity of amplification and calibrated by applying known constant currents through the 10,000-ohm input resistance, and measuring the vertical displacement of the resulting horizontal linear oscillograms. Linearity was observed up to 3.6  $\mu$ a. input.

## RESULTS AND DISCUSSION.

(i) *Evidence for Adsorption as Cause of Anomalies.*—Current-potential polarograms for the reduction of acridine in aqueous solutions at the d.m.e. were obtained at pH values ranging from 0 to 14, and at acridine concentrations in the range  $1-8 \times 10^{-4}$ M. Some typical examples are given in Figs. 3 and 4. At pH values above 3 it was necessary to include 5% of alcohol as co-solvent because of the low solubility of the free acridine in water. In general, the polarographic waves are markedly anomalous, and in no case was evidence found to support the general statement made by Breyer, Buchanan, and Duewell (*loc. cit.*) that acridine is reduced in two steps of equal height, although the shift of half-wave potential with change of concentration noted by these workers was confirmed. Multiple waves were of general occurrence and maxima were frequently encountered. Breyer, Buchanan, and Duewell's observation (*loc. cit.*) that the height of the first step remains constant throughout the concentration range  $10^{-4}$ — $10^{-3}$ M. was confirmed for pH values up to 4, but not for higher pH. This constant-height first step is shown in Fig. 3, where it is clear that it apparently consists of two smaller waves.

Inadequate buffering as a possible cause of the anomalous waves was suggested by the work



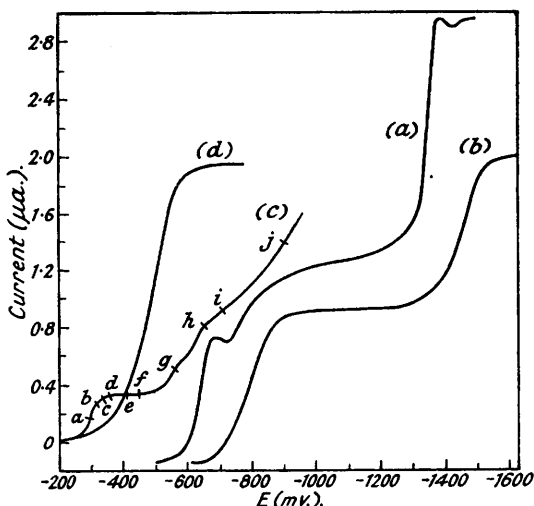
of Müller (*J. Amer. Chem. Soc.*, 1940, **62**, 2434), who showed that, when hydrogen ions are involved in electroreductions of organic materials, insufficient buffer may give rise to the appearance of two waves instead of one, as in the case of quinhydrone. However, the anomalies persisted even after greatly increasing the buffer-acridine concentration ratio or in 0.1N-hydrochloric acid supporting electrolyte, thus rendering this explanation of the anomalies improbable.

With higher concentrations of acridine, abnormal galvanometer oscillations were observed over a certain range of potentials, despite damping; the current increased very suddenly after the fall of a drop and then *decreased* slowly whilst the drop was still growing. At some dropping-mercury electrode potentials the current passed through several maxima during the life of a drop. We conclude that these effects are due to adsorption of electro-active material on the mercury surface. This conclusion is supported by the work of Albert, Goldacre, and Heyman (*J.*, 1943, 651), who studied the surface activities and partition coefficients of several acridines and found that unsubstituted acridine was the most hydrophobic and surface-active of the compounds examined.

To confirm that adsorption was the cause of the abnormalities, a run was done at pH 6.5 with  $4 \times 10^{-4}$ M-acridine in a buffer supporting electrolyte containing 60% (v/v) of alcohol, which was added in order to render the acridine less lyophobic. In the resulting polarogram almost all the

anomalies' disappeared; furthermore, the galvanometer oscillations became normal. Further increase of alcohol content to 70% and 75% led to completely normal polarograms. Since, however, such high alcohol contents tended to precipitate buffer salts, and it was desired to examine more closely the effect of varying alcohol content, 0.1N-hydrochloric acid was used as supporting electrolyte. A series of solutions, all containing 0.1N-hydrochloric acid and  $4 \times 10^{-4}$ M-acridine, but with alcohol contents ranging from 5 to 75%, was examined polarographically. The current-voltage curves obtained are reproduced in Fig. 5. Clearly 5% of alcohol is ineffective in preventing adsorption, giving a wave almost identical with that obtained without alcohol (Fig. 3). With increasing alcohol content the waves approach the normal form, reaching this at 60% alcohol. The multiple waves obtained in the absence of alcohol are thus not normal polarographic waves, but are due to adsorption effects. Curves *a* and *b* (Fig. 4) show that at pH 7.38, as also at other pH values, the addition of sufficient alcohol again removes the anomalous multiple waves. Finally, when these anomalous adsorption waves are suppressed by adding alcohol, there is good proportionality between wave-height and acridine concentration, rendering unacceptable Breyer, Buchanan, and Duewell's dimerisation hypothesis (*loc. cit.*), and there is then but a very small shift in half-wave potential with variation in acridine concentration.

FIG. 4.  
Effect of alcohol on acridine solutions.



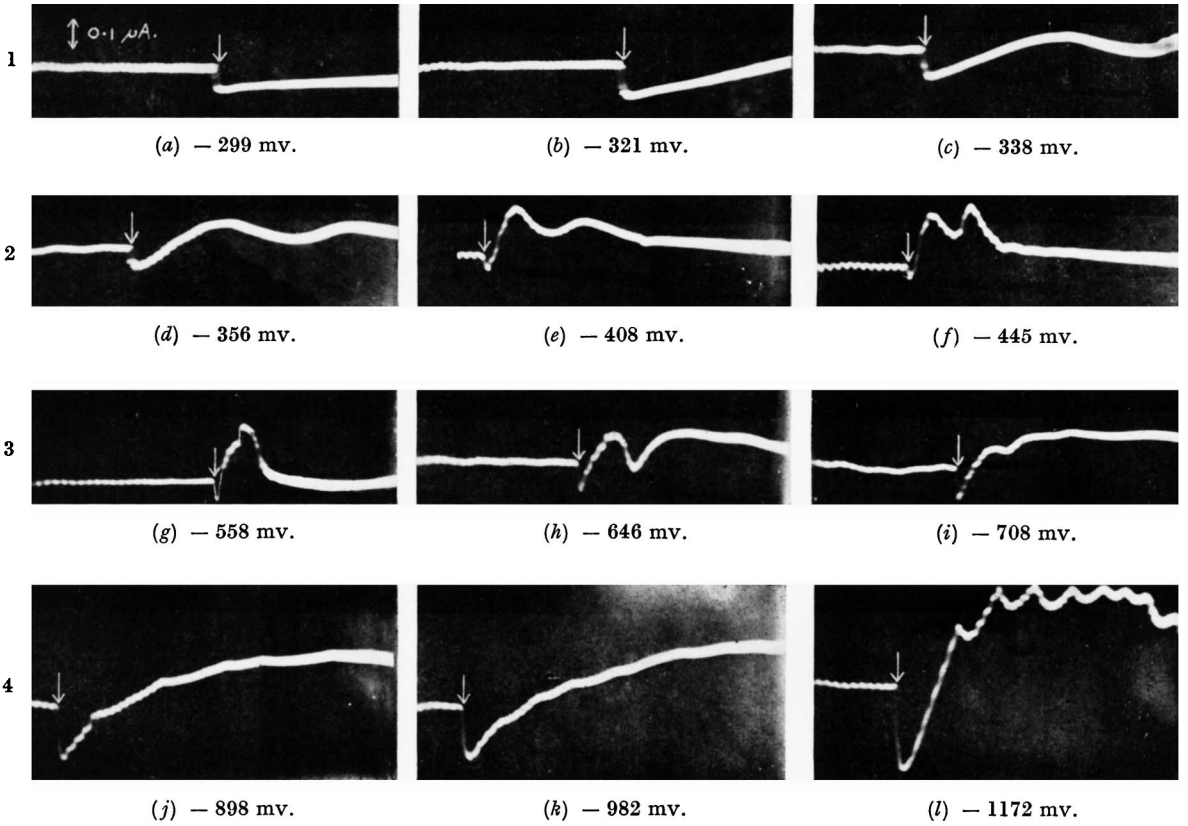
The current scale for curves (a) and (b) should be displaced 0.2  $\mu$ a. downwards from that shown.

- (a)  $4 \times 10^{-4}$ M-Acridine, pH 7.38; water.  
 (b) " " pH 8.29; 60% alcohol.  
 (c)  $10^{-3}$ M-Acridine, pH 1.0; water.  
 (d) " " pH 1.17; 60% alcohol.

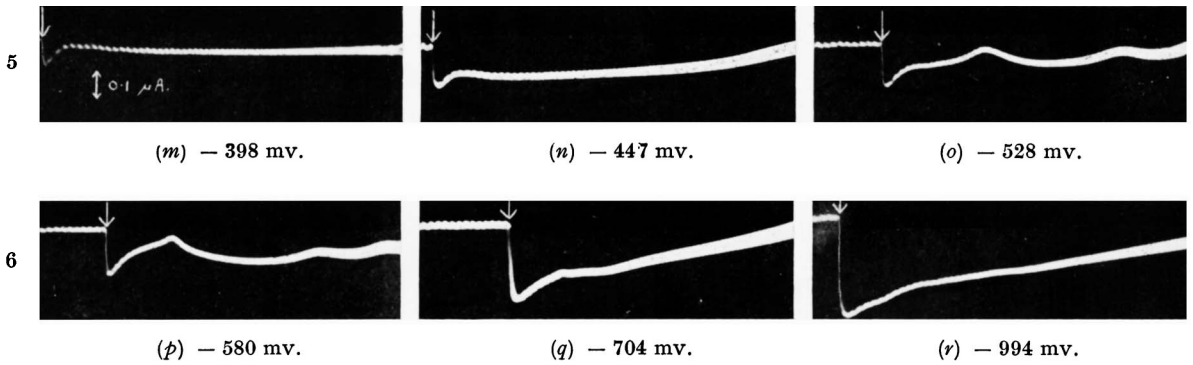
Since the abnormal galvanometer oscillations provided the first indication of adsorption effects, the current-time relationship during the earlier part (*ca.* 2 secs.) of a drop life-time was studied oscillographically in detail (see Experimental). Adsorption effects are likely to be greatest in this period, since the rate of surface increase is greatest when the mercury drop is small. This was confirmed before photographing the oscillograms, by inspecting visually both the early and the late parts of the drop life on the oscilloscope screen, the closing of the time-base switch being suitably delayed for this purpose; adsorption phenomena were confined mainly to the first two seconds of drop life. Fig. 6 (*a* to *l*) reproduces oscillograms showing the current-time relationship at various dropping-mercury electrode potentials for a solution containing  $10^{-3}$ M-acridine in an aqueous buffer at pH 1 (*i.e.*, 0.1N-HCl). It should be studied in conjunction with the correspondingly lettered polarogram (c) in Fig. 4. Curve (*a*) represents conditions at the bottom of the polarographic wave, where practically no reduction is occurring, and the current is essentially the residual or condenser current; the current-time relation is almost linear. Curves (*b*), (*c*), and (*d*) refer to the anomalous double adsorption wave in the polarogram (*cf.* Fig. 3); here the current passes through several maxima during a single drop life. This may possibly be due to adsorption on the growing mercury surface causing temporary blocking of the electrode surface

FIG. 6.

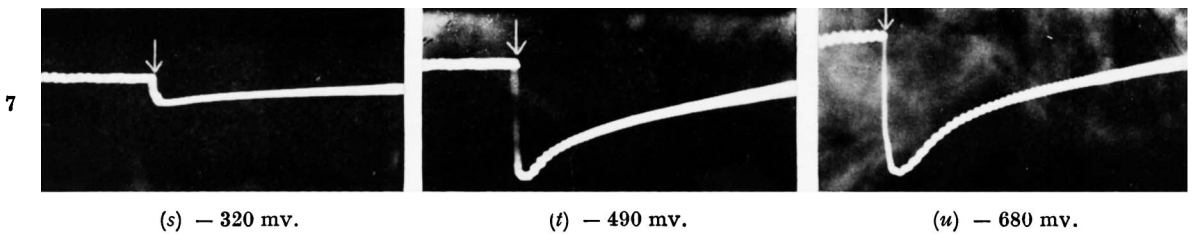
$10^{-3}\text{M}$ -Acridine in water at pH 1.0.



$10^{-3}\text{M}$ -Acridine in 50% alcohol at pH 1.0.



$10^{-3}\text{M}$ -Acridine in 75% alcohol at pH 1.0.

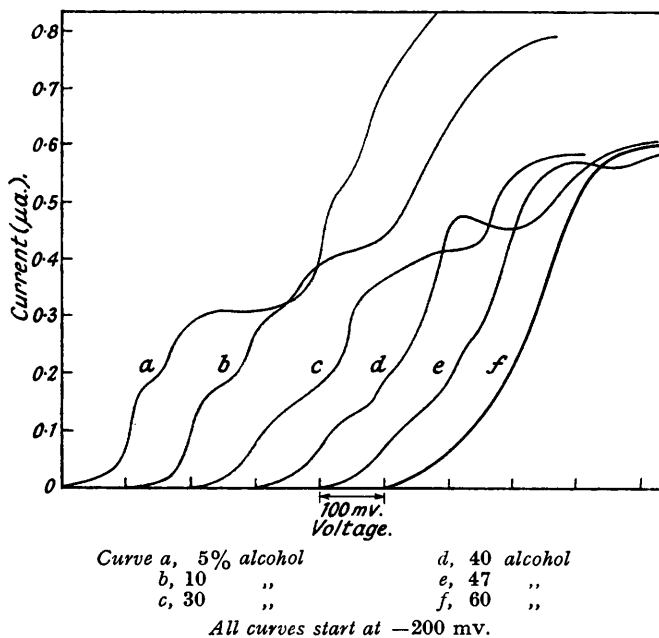


and also a fairly sudden local depletion of electro-active matter in solution, which is made good again by diffusion from the outer bulk solution after a short interval. At oscillogram (e) and in subsequent ones, a marked change is observed; immediately after the fall of a drop, the current rises abnormally rapidly, indicating that adsorption here enhances the reduction current. Lastly, at (f) the potential is sufficiently negative for hydrogen to be evolved, giving rise to the additional anomalous waves.

Fig. 6 (m to u) shows oscillograms obtained with the same system but with 50% (m to r) and 75% (s to u) of alcohol added. Apparently, 50% of alcohol reduces but does not completely remove the anomalies, while 75% of alcohol entirely eliminates them, permitting normal current-time curves to be obtained.

The adsorption anomalies with acridine in both polarograms and oscillograms are most pronounced in acid solutions; also the adsorption wave has a constant height throughout the concentration range  $10^{-4}$ — $10^{-2}$ M. at pH values up to but not exceeding 4. The  $pK_a$  value for acid-base ionisation of acridine is 5.6 in aqueous solution (Albert and Goldacre, *J.*, 1946, 706). This suggests that the acridinium ion rather than the free base is responsible for the anomalies. In support, results obtained with *N*-methylacridinium bromide, which of necessity exists only in the ionic form over the entire pH range, show that strong adsorption at the dropping-mercury

FIG. 5.  
Effect of alcohol on  $4 \times 10^{-4}$ M-acridine at pH 1.0 (in water).



electrode occurs in both acid and alkaline solutions, and is not completely eliminated by high concentrations (75%) of alcohol.

The constant-height adsorption wave previously mentioned resembles strongly the constant-height anomalous fore-waves obtained by Brdička (*Z. Elektrochem.*, 1942, 48, 278, 656) in the polarographic reduction of methylene-blue and of lactoflavin, which were ascribed to adsorption, confirmed by an oscillographic study. Since the results with lactoflavin are apparently complicated by slow rate-determining processes other than diffusion, we shall restrict our attention to Brdička's work on methylene-blue (*loc. cit.*, p. 278). He points out that, since the fore-wave occurs at potentials more positive than correspond to the main reduction wave, it follows that, the dropping-mercury electrode being treated as an inert-metal redox electrode of potential

$$E = E^{o'} + \frac{RT}{nF} \ln a_{ox.}/a_{red.} \dots \dots \dots (1)$$

(where  $a_{ox.}$ ,  $a_{red.}$  are the activities of oxidised and reduced forms of the organic electro-active material in the interface boundary layer near the mercury drop, and  $n$ ,  $E^{o'}$ ,  $R$ ,  $T$ , and  $F$  have

their usual significance), either  $a_{\text{ox}}$  is greater than, or  $a_{\text{red}}$  is less than the corresponding values for the bulk solution. The former alternative is inadmissible, but the latter is possible as a result of adsorption of reductant on the mercury, with consequent decrease in free energy. The constancy of height of the fore-wave for concentrations above a minimum and for acid pH values is ascribed to saturation of the mercury surface by adsorption of reductant; only after saturation is complete will an increased applied voltage cause the formation of unadsorbed reductant with an accompanying normal polarographic wave.

The preceding argument based on equation (1) being applied to our own results, it appears that the anomalous fore-waves (Figs. 3 and 5) are due to adsorption of the reduction product from cationic acridine, *i.e.* (since  $n = 1$ , as shown later), of semiquinone. Further, the distortions at more negative potentials which displace  $E$  to more negative values and which are responsible for the new feature appearing in Fig. 6 ( $e-l$ ) are due to adsorption of unreduced cationic acridine, which immediately removes electrons from the mercury.

Brdička assumed that the methylene-blue adsorption obeyed the Langmuir isotherm in the form

$$x = abc/(1 + bc) \quad \dots \dots \dots (2)$$

(where  $x$  = amount adsorbed in mol./cm.<sup>2</sup> at equilibrium concentration  $c$  mol./l.,  $a$  and  $b$  being constants). By combining this equation with equation (1) and the Ilkovič equation, he derived an equation giving  $E - E^{0'}$  as a function of the (mean) diffusion current  $\bar{i}$  and the adsorption current  $\bar{i}_a$  (the value of  $\bar{i}$  corresponding to the constant-height fore-wave). This last relation gives, with a reasonable choice of values for the constants involved, curves very similar to the experimental polarograms with adsorption fore-waves. By a similar treatment he showed that adsorption of oxidised form gives rise to an adsorption after-wave following the main reduction wave. From the equation he deduced that  $E^{0'}$ , the true half-wave potential at the given pH, is the potential at the midpoint of the main polarographic wave; this he found to be concentration-independent. Finally, introducing the mean rate of mercury surface growth, he deduced that the maximum value of  $x$  in equation (2), *i.e.*,  $a$ , is given by

$$a = \bar{i}_a/0.85nFm\bar{t}^{-\frac{1}{2}} \quad \dots \dots \dots (3)$$

where  $m$  is the rate of mercury flow in g./sec. and  $\bar{t}$  is the drop time in sec. For methylene-blue,  $a$  was evaluated from polarograms as  $1.62 \times 10^{-12}$  mol./cm.<sup>2</sup>, corresponding to an area per single molecule of about 100 Å.<sup>2</sup>. Applying these views to our results with acridine, we may conclude, from the occurrence of adsorption fore- and after-waves in the first and the second one-electron reduction step, respectively, that mainly semiquinone is adsorbed at all potentials and over the entire pH range examined; the adsorption is especially pronounced at low pH (<4) and not too negative potentials.

An important difference between the shapes of the adsorption fore-waves of methylene-blue and of acridine is that, whereas the former are sigmoid, the latter consist of two joined sigmoid portions. In this connection, it is of interest that Peacocke and Hinshelwood (*J.*, 1948, 2290) found that the adsorption of methylene-blue on *Bact. lactis aerogenes* followed a Langmuir type isotherm, but that of proflavine followed a sigmoid isotherm conforming to an equation of the form

$$x = abc^4/(1 + bc^4) \quad \dots \dots \dots (4)$$

which is suggested as arising from a co-operative effect due to strong inter-molecular fields, *i.e.*, the presence of adsorbed molecules on given sites facilitates the adsorption of similar molecules on neighbouring sites. It seems reasonable to suppose that equation (4) might apply to the adsorption of acridine on mercury, and to test this hypothesis the relation between  $E$  and  $\bar{i}$  (potential and polarographic current) was derived graphically with its aid as follows. Assuming a one-electron reduction (see below) and letting  $ox$  and  $s$  denote the concentrations of acridine and its semiquinone respectively in the boundary layer, we have by the Ilkovič equation

$$ox = (\bar{i}_a - \bar{i})/g \quad \dots \dots \dots (5)$$

where  $\bar{i}_a$  is the polarographic diffusion current and  $g$  is the proportionality factor between concentration ( $c$ ) and  $\bar{i}$  in the Ilkovič equation (*Coll. Czech. Chem. Comm.*, 1934, 6, 498)

$$\bar{i} = 605nD^{\frac{1}{2}}m\bar{t}^{\frac{1}{2}}c = ngc \quad \dots \dots \dots (6)$$

Following Brdička,  $\bar{i}$  is composed of two contributions, one from free semiquinone in the boundary layer, the other from adsorbed semiquinone, giving

$$\bar{i} = gs + \bar{i}_a bs^4/(1 + bs^4) \quad \dots \dots \dots (7)$$

where adsorption isotherm (4) has been used and  $\bar{i}_a$  is the adsorption saturation current. Hence, by equation (1) at 25°, concentrations being used instead of activities,

$$E - E^{\circ'} = 0.0591 \log (\bar{i}_a - \bar{i})/sg \dots \dots \dots (8)$$

With reasonable values  $\bar{i}_a = 0.75 \times 10^{-6}$  a.,  $\bar{i}_a = 0.25 \times 10^{-6}$  a.,  $g = 10^{-3}$ ,  $b = 10^{36}$ , this last relation may be plotted as  $E - E^{\circ'}$  against  $\bar{i}$  if  $s$  is evaluated for any given  $\bar{i}$  value by interpolation from a plot of  $\bar{i}$  against  $s$  corresponding to equation (7). The resulting polarogram was in fact very similar to the experimental curves, showing a double sigmoid fore-wave of height  $\bar{i}_a$ . Another test, in effect the reverse of the foregoing, was to take the experimental polarogram for  $4 \times 10^{-4}$ M-acridine at pH 1.925 (Fig. 3), evaluate from it  $E^{\circ'}$  (0.659 v.),  $\bar{i}_a$  ( $0.75 \times 10^{-6}$  a.),  $\bar{i}_a$  ( $0.25 \times 10^{-6}$  a.), and  $g$  ( $1.875 \times 10^{-3}$ ), and then by equation (8) evaluate  $s$  for various points on the adsorption wave and hence calculate the value of  $(\bar{i} - gs)/\bar{i}_a$ . This represents, following the reasoning upon which equation (7) is based, the current associated with and thus proportional to the adsorption, and a plot of this quantity against  $s$  is an adsorption isotherm. As expected, a sigmoid curve was obtained, confirming the approximate validity of (4). From (7) also, values of  $b$  were calculated, the fourth-power adsorption law being assumed; these values were not constant, however, ranging from  $0.6 \times 10^{36}$  to  $35 \times 10^{36}$ , showing that this law is only approximately correct. This is also apparent from the occasionally very complex adsorption waves encountered.

The replacement of the Langmuir isotherm (2) by the fourth-power isotherm (4) does not affect the result (3), which, applied to the polarograms for non-alcoholic solutions at pH 1.925 (Fig. 3,  $\bar{i}_a = 0.256 \times 10^{-6}$  a.) and pH 1.0 (Fig. 4c,  $\bar{i}_a = 0.277 \times 10^{-6}$  a.), with  $m = 0.00902$  g./sec.,  $t = 4.2$  secs., gives  $a = 1.162 \times 10^{-10}$  and  $1.169 \times 10^{-10}$  mol./cm.<sup>2</sup>, respectively. Thus, the area occupied per single molecule is  $1/6.03 \times 10^{23}$  or  $142.8$  and  $141.8$  Å.<sup>2</sup>, respectively (cf. Brdička's result: 100 Å.<sup>2</sup> for methylene-blue). (Structural formulæ indicate that the acridine molecule has a smaller area than that of methylene-blue, viz., approx. 50 Å.<sup>2</sup>.) This result, although expected to be only approximate, is certainly high.

Using abnormal galvanometer oscillations during polarographic reduction as a test, we have found other compounds structurally related to acridine, especially quinoline and methylene-blue, to exhibit adsorption effects. Methylene-blue adsorption was of course noted earlier by Brdička (*loc. cit.*).

(ii) *Semiquinone Formation.*—Breyer, Buchanan, and DUEWELL (*loc. cit.*) showed, by applying Ilkovič's equation, that in aqueous solutions at 0°, acridine was reduced in two one-electron stages, a semiquinone being the product of the first stage. Now adsorption, by affecting the normal proportionality between current and the one-sixth power of the time during the life of a drop, and also by imposing another factor in addition to the normally exclusive diffusion control of supply of electro-active matter to the d.m.e., may render inapplicable Ilkovič's equation (6) it is thus of interest to re-examine the values of  $n$  obtained from this equation. Table I gives values of  $n$  calculated for each step in our polarograms for aqueous solutions, equation (6) being used with the appropriate value of  $t$  for each half-wave potential, and with  $D^{\ddagger} = 2.75 \times 10^{-3}$  (calculated from the value of  $D$  at 0° and the temperature coefficient for  $\bar{i}_a$  or  $D^{\ddagger}$  as given by Breyer, Buchanan, and DUEWELL, *loc. cit.*). Where an adsorption fore-wave (or after-wave) occurs, its height has been added to that of the subsequent (or preceding) main wave (cf. Brdička, *loc. cit.*).

As was to be expected from the difficulty in estimating wave heights with any great accuracy, the first and the second step of a given polarogram were only approximately of equal height and  $\bar{i}_a$  was only approximately proportional to  $c$ . However, on the assumption that only two main waves are present, and the height of any adsorption wave being added to the appropriate main wave, the values of  $n$  which result show definitely that two one-electron steps are occurring. A more satisfactory test of the same kind, applied to the experiments in which 50—70% alcohol was present in the solutions, gave the results shown in Table II. Here there is little or no complication due to adsorption waves, and the polarograms consist essentially of two very nearly equal steps, except below pH 5 where the hydrogen wave overlaps the second one, making it unattainable experimentally. The value of  $D$  used for these alcoholic solutions was calculated by analogy with Gill's results (Ph.D. Thesis, London, 1947), which show that  $D^{\ddagger}$  decreased by  $38 \pm 2\%$  at 25° on addition of 50% alcohol to solutions of anthraquinone-1- and -2-sulphonic acid and 2-hydroxy- and 1:2-dihydroxy-anthraquinone, and that for the 2-hydroxy-derivative it was practically constant over the range 50—70% alcohol (cf. Shreve and Markham, *loc. cit.*, who found that  $\bar{i}_a$  for *p*-nitroaniline is almost constant for 46—58% alcohol and about 34% lower than in water, and that  $D^{\ddagger}$  is always proportional to  $\bar{i}_a$  since  $m^{\ddagger}/j^{\ddagger}$  is practically inde-



TABLE I.

Results for reduction of acridine in aqueous solutions.

pH.	c, millimol./l.	First step.				Second step.				Remarks.
		$\bar{i}_d$ ( $\mu$ a.).	n.	$-E_a$ (mv.).	$-E_1$ (mv.).	$\bar{i}_d$ ( $\mu$ a.).	n.	$-E_2$ (mv.).	$-E_b$ (mv.).	
1.0	0.2	0.4	1.01	344	689	—	—	—	—	} Hydrogen wave overlaps 2nd step
	0.4	0.8	1.01	315	650	—	—	—	—	
1.925	0.1	0.2	0.99	351	—	—	—	—	—	} " "
	0.2	0.38	0.96	335	669	—	—	—	—	
	0.4	0.76	0.96	313	660	—	—	—	—	
	0.8	1.39	0.88	291	710	—	—	—	—	
3.95	0.2	0.4	1.00	350	680	—	—	—	—	} " "
	0.4	0.74	0.95	340	646	—	—	—	—	
5.58	0.1	0.2	1.01	428	—	0.24	1.22	1313	—	} 5% Alcohol added
	0.2	0.4	1.01	435	720	0.42	1.07	1320	1435	
	0.4	0.7	0.89	470	690	0.78	0.99	1338	1440	
7.38	0.2	0.42	1.06	603	856	0.40	1.01	1329	—	} " "
	0.4	0.71	0.97	637	805	0.80	1.01	1324	—	
9.07	0.2	0.42	1.06	765	985	0.36	0.91	1330	—	} " "
	0.4	0.63	0.80	778	950	0.75	0.95	1346	—	
11.0	0.1	0.24	1.22	865	1060	0.20	1.01	1330	—	} " "
	0.2	0.42	1.06	905	1147	0.36	0.91	1330	—	
	0.4	0.55	0.70	919	1150	0.65	0.82	1333	1440	
13.0	0.1	0.23	1.17	1054	1228	0.20	1.01	1318	—	} " "
	0.2	0.42	1.06	1082	1242	0.42	1.06	1325	1425	
	0.4	0.54	0.68	1092	1236	0.70	0.89	1340	1430	

TABLE II.

Results for reduction of acridine in alcoholic solutions.

pH of buffer :		C, millimol./l.	First step.			Second step.			Alcohol, %.
in H <sub>2</sub> O.	with 50% EtOH.		$\bar{i}_d$ ( $\mu$ a.).	n.	$-E_1$ (mv.).	$\bar{i}_d$ ( $\mu$ a.).	n.	$-E_2$ (mv.).	
1.0	1.17	0.2	0.27	1.10	430	—	—	—	60
		0.4	0.56	1.14	437	—	—	—	60
		0.8	1.22	1.24	455	—	—	—	60
		0.4	0.60	1.22	455	—	—	—	50
		0.4	0.61	1.24	435	—	—	—	60
		0.4	0.57	1.19	453	—	—	—	50
		1.0	1.90	1.54	477	—	—	—	60
1.925	2.36	0.2	0.30	1.22	410	—	—	50	
3.95	4.81	0.4	0.53	1.07	520	—	—	60	
5.58	6.51	0.4	0.44	0.90	653	0.60	1.22	1410	50
		0.4	0.50	1.02	695	0.50	1.05	1467	70
		0.4 *	0.38	0.99	718	0.31	0.96	1485	70
7.38	8.29	0.4	0.53	1.07	792	0.53	1.00	1447	55
9.07	10.39	0.4	0.55	1.11	935	0.50	1.04	1428	50
11.0	12.00	0.4	0.54	1.09	1032	0.50	1.04	1457	55
13.0	13.39	0.4	0.55	1.11	1160	0.56	1.17	1425	55

\* Temp. 0° c.

pendent of alcohol content or pH). At 25°, we therefore used  $D^{\frac{1}{2}} = 1.71 \times 10^{-3}$ , and at 0°,  $D^{\frac{1}{2}} = 1.33 \times 10^{-3}$ , for 50—70% alcohol. The results, although affected by the uncertainty in  $D$ , indicate clearly the existence of two one-electron steps.

In spite of this fact, a graph of  $E$  against  $\log(\bar{i}_d - \bar{i})/\bar{i}$  for  $4 \times 10^{-4}$ M-acridine in a pH 5.58 buffer containing 70% alcohol, at 25°, although linear, has a slope of 98 mv. for the first step and 66 mv. for the second step, as compared with 59 mv. expected for a reversible one-electron process. With lower alcohol content, e.g., with  $8 \times 10^{-4}$ M-acridine in a buffer at pH 1.0 containing 60% alcohol, the graph is somewhat curved with a slope of about 100 mv. for the first step. Thus the reduction at the d.m.e. is not thermodynamically reversible. It follows that these polarographic potentials cannot be treated strictly as reversible redox potentials, but are more akin to the apparent redox potentials (A.R.P.) of Conant *et al.* (*J. Amer. Chem. Soc.*, 1923, 45, 1047; 1924, 46, 1254; 1926, 48, 2468, 3178; 1930, 52, 407; *Chem. Reviews*, 1926, 3, 1). It

is, however, still permissible to attach qualitative significance to values of the semiquinone formation constant  $K$ , defined by

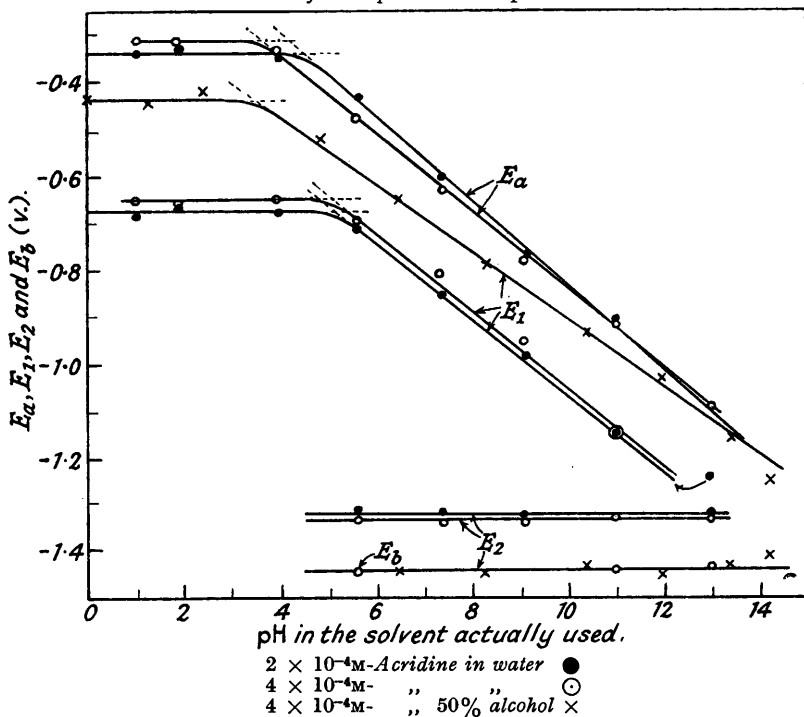
$$K = s^2/(\text{red.})(\text{ox.}) \dots \dots \dots (9)$$

("red." represents the concentration of fully reduced dihydroacridine), when this is calculated by treating the half-wave potentials  $E_1$  and  $E_2$  of the two one-electron steps as if they were reversible potentials, and substituting in the equation (Michaelis, *Ann. N.Y. Acad. Sci.*, 1940, 40, 39)

$$\sqrt{K} = 10^{E_i/0.0591} - 3 \times 10^{-E_i/0.0591} \dots \dots \dots (10)$$

where  $E_i$ , the index potential, is equal to  $\frac{1}{2}(E_1 - E_2)$ . Geake's more detailed procedure (*Trans. Faraday Soc.*, 1938, 34, 1395) for calculating  $K$  is inapplicable because of the lack of the necessary symmetry of the polarograms about the midpoint.

FIG. 7.  
*Half-wave potentials vs. pH.*



In order to utilise equation (10), values of  $E_1$  and  $E_2$  for  $2 \times 10^{-4}\text{M-}$  and  $4 \times 10^{-4}\text{M-}$  acridine in water (Table I) and  $4 \times 10^{-4}\text{M-}$  acridine in 50% alcohol (Table II) were plotted against measured pH, as in Fig. 7. For the alcoholic solutions, some data referring to acridine or alcohol concentrations differing slightly from those stated above had to be used; however, Table II shows that this is not likely to cause serious error, since a variation from 45% to 55% alcohol, or from  $2 \times 10^{-4}\text{M-}$  to  $8 \times 10^{-4}\text{M-}$  acridine, affects  $E_1$  or  $E_2$  by a few centivolts at most. From these plots, smoothed values of  $E_1$  and  $E_2$  were derived by drawing the best-fitting straight lines through experimental points, and these were substituted in equation (10) to give the  $K$  values listed in Table III. In most cases, the large values of  $E_i$ , due to the great separation of the two reduction waves, rendered the second term of equation (10) negligible in comparison with the first.

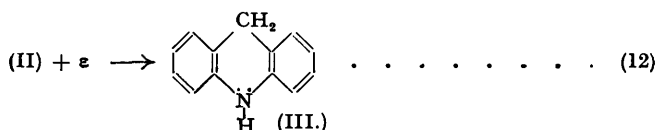
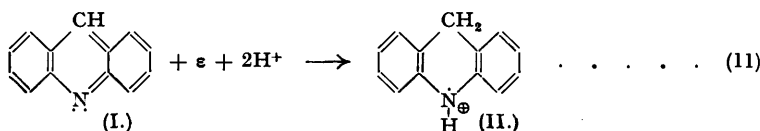
These large  $K$  values (except at pH 13 in water) indicate that the half-wave potentials  $E_1$  and  $E_2$  approximate closely to the apparent standard redox potentials at any given pH for the two one-electron processes; it is easily shown that the former quantities differ from the latter by  $\frac{RT}{F} \ln \left( \sqrt{\frac{3}{K} + \frac{1}{4}} + \frac{1}{2} \right)$  which approaches zero for large values of  $K$ . Fig. 7 being interpreted

TABLE III.  
Values of K at various pH values.

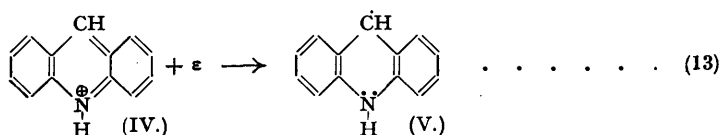
Buffer *	{ In H <sub>2</sub> O	3.94	5.58	7.38	9.07	11.04	13.0	14.0
pH	{ In 50% EtOH	4.81	6.51	8.29	10.39	12.00	13.39	14.27
$E_1$ (volts)	$2 \times 10^{-4}$ M-Acridine in H <sub>2</sub> O	—	0.305	0.237	0.168	0.092	0.044	—
	$4 \times 10^{-4}$ M-Acridine in H <sub>2</sub> O	—	0.325	0.252	0.180	0.100	0.052	—
	$4 \times 10^{-4}$ M-Acridine in 50% EtOH	0.457	0.395	0.322	0.262	0.200	0.150	0.120
$\log \sqrt{K}$	$2 \times 10^{-4}$ M-Acridine in H <sub>2</sub> O	—	5.16	4.01	2.84	1.56	0.70	—
	$4 \times 10^{-4}$ M-Acridine in H <sub>2</sub> O	—	5.50	4.26	3.05	1.69	0.88	—
	$4 \times 10^{-4}$ M-Acridine in 50% EtOH	7.74	6.68	5.45	4.43	3.38	2.60	2.03

\* The pairs of pH values above refer to the same buffer mixture in the two different solvents.

on this basis, the zero slope of the graph of  $E_2$  against pH over the accessible pH range indicates that the second reduction step involves no hydrogen ions. On the assumption (cf. Lingane *et al.*, *J. Amer. Chem. Soc.*, 1943, **65**, 1348) that the final reduction product is acridan (III), with two more hydrogen atoms than acridine (I), it would seem that, for pH 5—13 (3.6—13 in alcoholic solutions), the first step should involve two hydrogen ions. Thus the following scheme for the two-stage reduction is plausible in this pH range :



Below about pH 5.1 in water or 3.6 in 50% alcohol,  $E_1$  becomes constant, independent of pH, indicating that the first reduction step now involves no hydrogen ions. These transition pH values accord approximately with the  $\text{p}K_a$  values 5.6 and 4.11 for the acid-base ionisation of acridine in water and 50% alcohol, respectively, at  $20^\circ \pm 5^\circ$  (Albert and Goldacre, *J.*, 1946, 706). At pH below  $\text{p}K_a$ , acridine will exist mainly in the acid or ammonium ion form (IV). Thus in this pH region, the first reduction step becomes



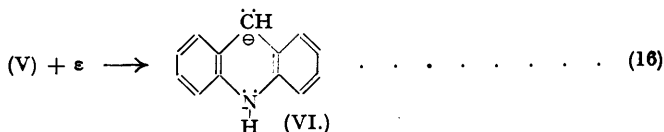
At such acid pH, the second step probably becomes



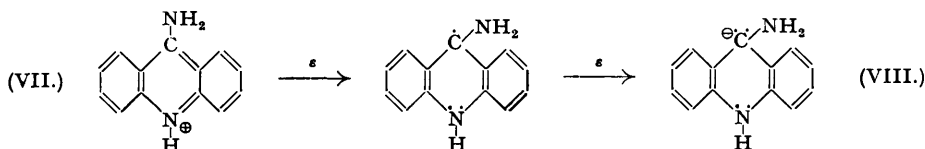
and accordingly the graph of  $E_2$  against pH should have a slope of 0.059 v. per pH unit at pH below  $\text{p}K_a$ . This is, however, unverifiable, since the hydrogen-deposition wave overlaps the acridine reduction wave at pH below about 5.

There are, nevertheless, objections to the reduction scheme of equations (11) and (12). The  $E_1$ -pH graph should, according to equation (11), have a slope of 0.118 v. per pH unit, while the actual slopes are 0.080, 0.084, and 0.071 v. for  $2 \times 10^{-4}$ M-aqueous,  $4 \times 10^{-4}$ M-aqueous, and  $4 \times 10^{-4}$ M-alcoholic (50%) solutions of acridine, respectively, at pH 5—13. These slopes are closer to the value of 0.059 v. for a step involving only one hydrogen ion, the remaining discrepancy being ascribable to the irreversibility of the reduction; alcohol-water junction potentials apparently reduce the discrepancy. Further, it is unlikely that the semiquinone would exist in the cationic or acidic form (II) at pH above 5 (equation 11), and in the non-ionic or basic form (V) at more acid pH values (equation 13); the reverse behaviour would be expected if the semiquinone could undergo acid-base dissociation in this way. In fact, there is no evidence of such dissociation, since this would cause a fairly sharp decrease in the slope of the  $E_1$ -pH curve or increase in the slope of the  $E_2$ -pH curve when the pH rose above  $\text{p}K_s$ , where  $K_s$  is the

hypothetical acid constant of the semiquinone; neither effect is observed. In view of these objections, the following alternative reduction scheme may be proposed for pH above 5 in water or 3.6 in 50% alcohol :



Support for this scheme is provided by some results (to be published) which we have obtained for the polarographic reduction of 5-aminoacridine; this gives two one-electron reduction steps, the half-wave potentials of which are both independent of pH over a considerable alkaline range lying below  $pK_a$  for the unreduced substance, indicating that the latter when mainly in the cationic form (VII) undergoes the following two-stage reduction not involving hydrogen ions, and gives an anionic product (VIII) similar to (VI) :



Substances (V) and (VI) might be expected to react with unreduced acridine (see below) and abstract a proton from water, respectively; such processes, if slow, could explain the irreversibility of the two reduction steps (15) and (16). Processes similar to (16), not involving hydrogen ions, and leading to substances like (VI) which probably take up a proton from a water molecule to form a neutral final product, have been postulated for the second of two one-electron reduction steps of phenolphthalein in its red quinonoid form (Kolthoff and Lehmicke, *J. Amer. Chem. Soc.*, 1948, 70, 1879), of 8-hydroxyquinoline (Stock, *J.*, 1949, 586), of cinchonic acid (Casimir and Lyons, *J.*, 1950, 783), and possibly of quinoline-8-carboxylic acid (Stock, *J.*, 1949, 763).

Let T, T<sup>+</sup> denote the un-ionised and the ionised form of acridine, respectively, and S the sole form of semiquinone (V) postulated in equations (13)–(16). For a reversible process corresponding to equation (15), with standard redox potential  $E_{15}^0$ , the redox potential (in v.) at 25° is given by

$$E = E_{15}^0 - 0.059 \log [S]/[T][H^+] \quad \dots \dots \dots (17)$$

For the ionisation equilibrium of acridine we have

$$[T^+] = [T][H^+]/K_a$$

and for the total concentration of unreduced acridine

$$[Ox] = [T] + [T^+] = [T]\{1 + [H^+]/K_a\}$$

Substituting for [T] in terms of [Ox] in equation (17), we have

$$E = E_{15}^0 + 0.059 \log K_a - 0.059 \log [S]/[Ox] - 0.059 \log ([H^+] + K_a)/[H^+]$$

At the half-wave potential for reduction (15), [Ox] = [S] and  $E = E_1$ , so

$$E_1 = E_{15}^0 + 0.059 \log K_a - 0.059 \log ([H^+] + K_a)/[H^+] \quad \dots \dots \dots (18)$$

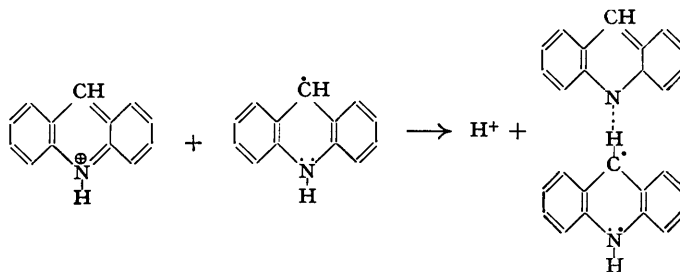
If this treatment had been based on equation (13), the same result would have been obtained except that  $E_{13}^0$  would replace  $E_{15}^0 + 0.059 \log K_a$ . That these are equal follows from a consideration of the free-energy changes in (13), (15), and the ionisation of acridine. With a suitable choice of values for  $E_{13}^0$  and  $K_a$  (e.g., -0.435 v. and  $10^{-3.6}$  respectively for 50% alcoholic solutions), equation (18) when plotted as  $E_1$  against pH reproduces the experimental curve (Fig. 7) fairly well, except that the slope of the inclined portion is 0.059 v. A similar treatment based on equation (11), the sole form of semiquinone assumed to be (II), leads to

$$E_1 = E_{11}^0 + 0.059 \log K_a - 0.059 \log ([H^+] + K_a)/[H^+]^2$$

which gives an  $E_1$ -pH graph consisting of two inclined portions, one at pH below  $pK_a$  with slope 0.059 v., the other above  $pK_a$  with slope 0.118 v., contrary to the experimental results.

In Table I are given values of the half-wave potentials  $E_a$  and  $E_b$  for the adsorption fore-wave of the first reduction step and the adsorption after-wave of the second step, respectively. These

are plotted against pH in Fig. 7. The  $E_a$ -pH curve closely resembles the  $E_1$ -pH curve, with a zero slope portion at low pH and a one-hydrogen-ion slope at higher pH, indicating that the same reduction processes (13) and (15) occur over the potential regions corresponding to the zero-slope and the first main reduction wave. Similarly, the  $E_b$ -pH and  $E_2$ -pH graphs both have zero slope. However, the transition pH between zero and non-zero slopes is lower for  $E_a$  (4.5 and 3.7 for  $2 \times 10^{-4}M.$  and  $4 \times 10^{-4}M.$ , respectively) than for  $E_1$  (5.1). This indicates that adsorption of semiquinone has in some way lowered the effective  $pK_a$  of unreduced acridine. A possible mechanism, suggested by the large area occupied by each semiquinone molecule at adsorption saturation and by the co-operative adsorption isotherm which applies approximately, is interaction of acridine with semiquinone to release a hydrogen ion from the former, whereupon the two molecules may link by hydrogen bonding; the net effect is co-adsorption of acridine with semiquinone, together with enhanced acid ionisation of the acridine :



The high apparent  $K$  values in Table III suggest that the semiquinone (V) has a great stability against disproportionation into acridine and acridan. This stability is enhanced by adding alcohol to the solvent (cf. Burstein and Davidson, *Trans. Electrochem. Soc.*, 1941, **80**, 175) in keeping with the non-ionic nature of (V). It may be ascribed to resonance among various structures in which the odd electron may be situated as in (V), or in either of the benzene rings in positions ortho or para to the point of attachment to the nitrogen atom; allowing for Kekulé benzene-ring resonance, there are thus 16 contributory structures, as compared with only 4 each due to Kekulé resonance, for acridine and acridan.

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