THE DETERMINATION OF n-VALUES FOR SOME AMINOACRIDINES BY CONTROLLED POTENTIAL COULOMETRIC REDUCTION

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The reduction of some aminoacridines has been investigated by controlled potential coulometry. The number of electrons involved in the first reduction stage has been determined by a graphical treatment of the current-time values obtained using a divided electrolysis cell. With the exception of 5-aminoacridine the results confirm the values obtained indirectly by Kaye[5].

THE method of controlled potential electro-reduction at a stirred mercury cathode was introduced by Lingane and is discussed in his paper[1] and in his book[2]. This procedure was developed primarily as an analytical method but it can also be adapted to determine the number of electrons involved (n-value) in the reduction process at the cathode[1].

Kaye and Stonehill[3] in their investigations of the polarographic reduction of some aminoacridines concluded that at the dropping mercury electrode the reductions take place in two one-electron stages, with the intermediate formation of a stable semiquinone radical. The n-value they obtained was deduced by an application of the Ilkovic equation[4], making assumptions about the value of the diffusion coefficient of the aminoacridine in ethanol.

Since Kaye's theory of the antibacterial action of the aminoacridines[5] is based on this interpretation, it was thought to be of importance to determine the value of n by an independent method.

METHODS

The circuit and apparatus described by Lingane[1,6] was used in preliminary experiments. In these a controlled potential, obtained from a potential divider, was applied to the electrolysis cell, consisting of a silver–silver chloride anode and a mechanically stirred mercury cathode of about 30 sq. cm. surface area. The potential of the cathode was measured with reference to a saturated calomel electrode and the applied voltage was adjusted manually so as to maintain this potential at an appropriate value. The quantity of electricity consumed in the reduction of a known amount of oxidant was measured by means of a hydrogen-oxygen gas coulometer in series with the reduction cell. The coulometer contained potassium sulphate (0·5M) as electrolyte. The corrected volume of mixed gas per coulomb was determined and its value found to agree with that obtained by Lingane[1].

According to Lingane [1,6] the current should decrease exponentially with time during the course of the reduction (equation 1).

$$I = I_0 10^{-k't} \qquad (1)$$

where I is the current measured at time t, I_0 is the initial current and k′ is a constant depending on the cell geometry, rate of stirring and temperature. However when 5-aminoacridine was reduced in this cell the current fell to a constant value of approximately half the initial value and a graph of log I against time was not linear, indicating that the fall was not in accordance with equation 1 (Fig. 1).

It was considered that this was due to reoxidation of the reduced material at the anode. To eliminate this possibility a divided cell was constructed, similar to that described by Lingane (ref. [2], p. 478), in which

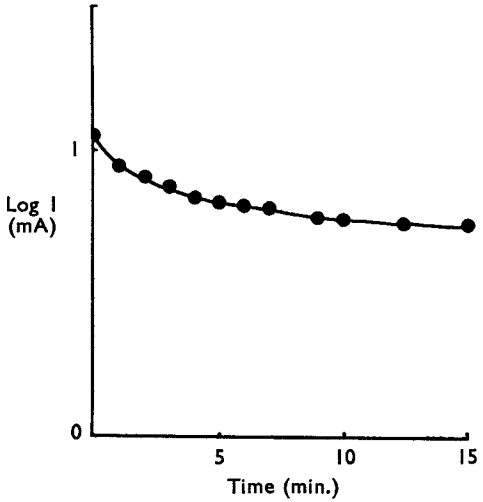

FIG. 1. Decay of current with time (undivided cell) 100 mg. of 5-aminoacridine in alcoholic buffer. 1300 mV *v* SCE.

anolyte and catholyte are separated by a sintered glass disc covered with an agar plug. On repeating the reduction of 5-aminoacridine in this cell, at a potential of 1,300 mV *versus* a saturated calomel electrode, corresponding to the top of the first step of the polarographic wave of 5-aminoacridine[3], the current was observed to fall exponentially as required by equation 1, and the calculated n-value was found to be approximately two.

When 2,8-diaminoacridine was examined in a similar manner, n-values of 0·81, 1·0, and 1·08 were found.

In all these experiments the precise control of potential caused difficulty, and in addition each reduction was time consuming (90–120 min.). In the early stages of each reduction it was found that the desired potential could not be achieved, due to the variability of the voltage drop across the gas coulometer. Any increase of the applied voltage beyond a certain point had little effect on the potential of the stirred cathode. On shorting out the coulometer the desired potential could be achieved without difficulty.

MacNevin and Baker[7] have pointed out that integration of equation 1 will yield the necessary quantity of electricity and experiments were

made with the object of investigating the regularity with which the current decayed, with a view to using a graphical method of current integration. These revealed that stirring rate was the only important factor influencing the rate of a given reduction (i.e., the value of k′ in equation 1). Stirring by means of paddle proved to be too irregular to give a good linear graph of log I against time. On replacing the paddle by a slotted polythene disc with a flat sided polythene covered magnetic paddle fused to it, the current was found to decay smoothly with time provided the stirring rate was not excessive (Fig. 2).

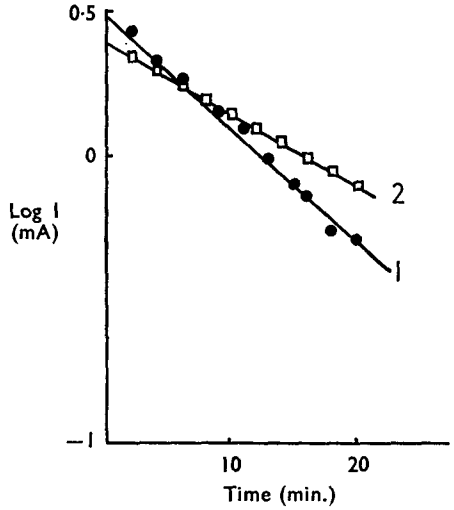

FIG. 2. Decay of current with time (divided cell), 5 mg. proflavine base in alcoholic buffer, 1200 mV *v* SCE. 1, rapid stirring; 2, moderate stirring.

For convenience in evaluation the concept of "half time" of the reaction was used.

$$\text{Since } I = I_0e^{-kt} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

when $t = t_{\frac{1}{2}}$ (the time required for the current to fall to half of its original value, i.e., the time required to reduce half of the oxidant), then

$$\tfrac{1}{2} = e^{-kt_{\frac{1}{2}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

$$\text{when } k = 0{\cdot}693/t_{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

For half reduction the quantity of electricity required, $Q_{\frac{1}{2}}$, is given by

$$Q_{\frac{1}{2}} = \int_0^{t_{\frac{1}{2}}} I\,dt = \int_0^{t_{\frac{1}{2}}} I_0e^{-kt}\,dt$$
$$= (I_0e^{-kt_{\frac{1}{2}}})/k$$

and introducing (3) and (4)

$$Q_{\frac{1}{2}} = I_0t_{\frac{1}{2}}/0{\cdot}693 \times 2 \quad .. \quad .. \quad (5)$$

For reduction of the whole of the sample

$$Q = I_0t_{\frac{1}{2}}/0{\cdot}693 \quad .. \quad .. \quad .. \quad (6)$$

When t is in seconds and I is in amperes Q is in coulombs, hence from Faraday's Laws,

$$n = \frac{I_0 t_{\frac{1}{2}} M}{0{\cdot}693 \times 96{,}500 \times w} \quad .. \quad .. \quad .. \quad (7)$$

when w is the weight in g. of reducible substance of molecular weight M.

From the graph of log I against t, obtained by reading I at 1 minute intervals, I_0 and $t_{\frac{1}{2}}$ can be determined. The reduction period was extended to cover about two half times. The circuit and apparatus used for this work are shown in Figure 3.

A Pye "Scalamp" galvanometer was used for current measurement. It was suitably shunted so that 1 cm. deflection corresponded to 1 mA on

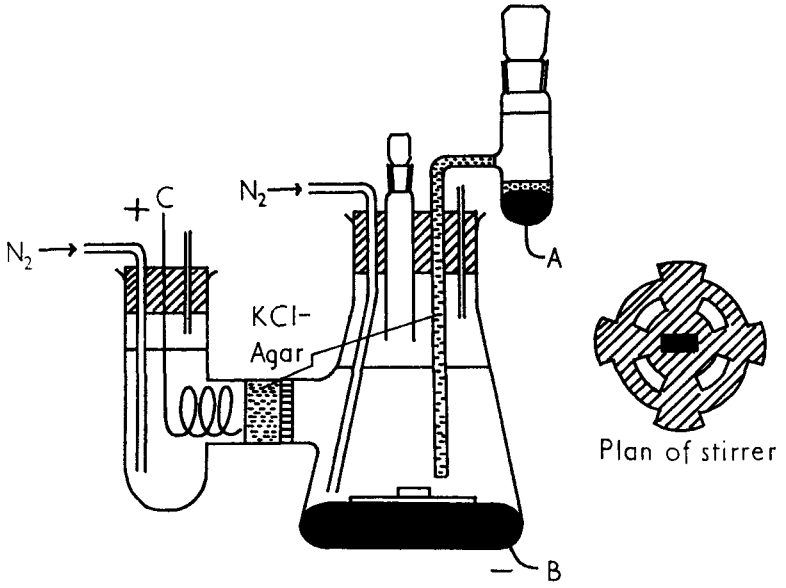

FIG. 3, *a*. Divided cell assembly.

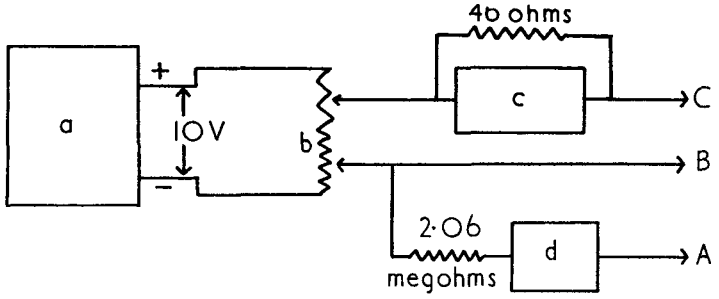

FIG. 3, *b*. Circuit diagram. a, direct current source (Labgear Eliminac); b, potential divider; c, galvanometer for current measurement; d, galvanometer for potential measurement.

the 0·001 range. A Cambridge "Spot" galvanometer was used for measurement of potential, by placing in series a suitable resistance. The current required to give a full-scale deflection did not exceed 1μA, which was too small to affect materially the calculated n-value, and did not alter the potential of the large capacity calomel electrode from that measured with a valve voltmeter.

The solvents used in this work were those which Kaye and Stonehill[3] found to give satisfactory polarographic waves for aminoacridines. Reducible impurities were first removed from the solvent by subjecting 150 ml. to the working potential in the reduction cell in the presence of a stream of oxygen-free nitrogen until the current fell to a constant value of

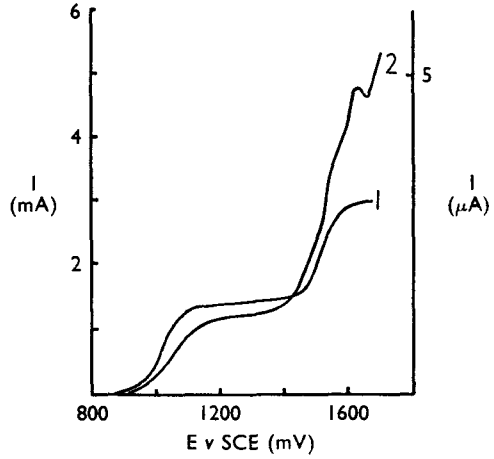

FIG. 4. 1, Polarogram of 2×10^{-4} M proflavine base in alcoholic buffer (microamp scale). 2, Current voltage curve at stirred mercury cathode for 1×10^{-4} M proflavine base in the same alcoholic buffer (milliamp scale).

0·03 to 0·05 mA. It was assumed that this "background" current remained constant throughout the subsequent reduction, and its value was subtracted from the observed total current before plotting graphically. A full discussion of background corrections has been given by Meites and Moros[8].

It has been pointed out[1,6] that the polarographic method forms a reliable pilot technique to establish the optimum conditions of potential, composition of supporting electrolyte and concentration, for coulometric analysis. In this work however, to establish the optimum value of the working potential, current–voltage curves for the electroreduction of the aminoacridines were obtained by dissolving about 1 mg. of the substance in 150 ml. of reduced ethanolic buffer and measuring the current at 40 mV intervals of potential. This procedure was completed as rapidly as possible to minimise the effect of concentration changes produced by the electrolysis. The current-voltage curve so obtained for 2,8-diaminoacridine is shown in Figure 4, together with a polarogram of the same solution. These curves show that a more negative potential is

required to attain the top of the first step, than is indicated by the polarogram. This was found to be general for the aminoacridines.

As a further precaution a series of current-voltage curves was prepared using quntities of aminoacridine ranging from 1 to 10 mg. Such a series is shown in Figure 5 and it is clear that with increasing concentration definition of the working potential becomes more difficult. Coulometric determinations were therefore made using samples of aminoacridine not greater than 5 mg.

In each experiment 1–5 mg. of the aminoacridine was dissolved in the prepared buffer solution. The control potential was applied and a stop

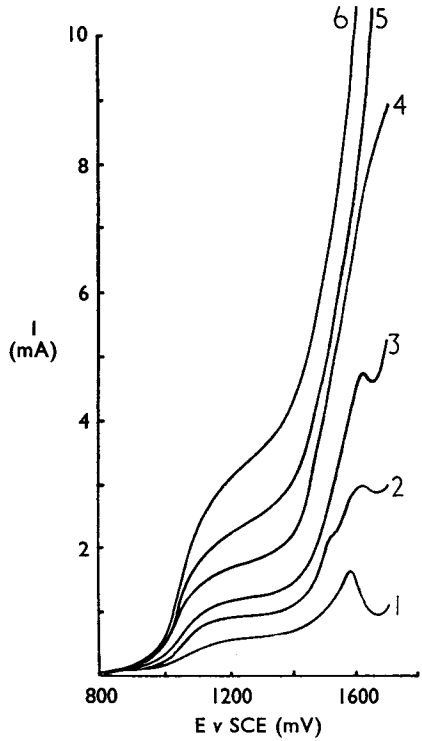

FIG. 5. Current voltage curves for proflavine base in alcoholic buffer (obtained by successive addition of 1, 1, 1, 2, 2 and 3 mg. to a total of 10 mg.).

clock was started simultaneously. Readings of the current were made at 1 minute intervals, the potential being maintained throughout by manual adjustment of the potential divider. Readings were taken until the current had fallen to about one-quarter of its original value (20–30 min.). The results were plotted on semi-log paper. Although replicate determinations on similar samples did not always yield the same values of initial current and half time (Fig. 2), the product of these two factors (see eqn. 6) remained fairly constant.

The quantities of 1-aminoacridine, 2-aminoacridine, 3-aminoacridine and 4-aminoacridine available did not permit more than three or four

determinations to be made, but some indication of the precision of the method is given by the series of n-values obtained for 2,8-diaminoacridine. It is probably not better than $\pm$ 10 per cent. However since the object of the present work was to evaluate n-values it was considered that this accuracy was quite adequate when n is one or two.

Results

A summary of these is given in Table I.

TABLE I

Summary of experimental conditions and results obtained for aminoacridines

Substance	Potential mV	wt. mg.	M.wt.	I_o mA	$t_{\frac{1}{2}}$ min.	n-value
1-aminoacridine	1,075	5·0	194	3·00	10·5	1·10
"	1,075	5·0	194	3·60	8·7	1·09
2-aminoacridine	1,040	2·4	194	1·35	10·7	1·10
"	1,040	1·14	194	0·70	9·8	1·05
3-aminoacridine	1,020	1·2	194	0·58	11·0	0·93
"	1,020	1·2	194	0·72	10·3	1·08
4-aminocridine	1,100	1·05	194	0·52	10·8	0·93
"	1,100	0·95	194	0·54	10·0	0·99
5-aminoacridine hydrochloride	1,350	0·98	230·5	0·53	19·0	2·13
"	1,350	4·6	230·5	1·92	23·0	1·98
2,7-diaminoacridine	1,000	5·0	209	1·55	16·8	0·98
"	1,000	4·8	209	2·32	11·2	1·0
2,8-diaminoacridine	1,200	4·0	209	1·71	11·4	0·91
"	1,200	3·12	209	1·60	10·9	1·04
"	1,200	4·0	209	1·82	11·1	0·95
"	1,200	5·1	209	2·27	11·5	0·96
"	1,200	5·0	209	3·20	7·6	0·91
"	1,200	5·2	209	2·48	11·9	1·06
"	1,200	5·0	209	4·12	6·6	1·02

Discussion

Two conclusions emerge from the results. With the exception of 5-aminoacridine, the first step of the current-voltage curve, which corresponds to the first step of the polarographic wave of the aminoacridines, is shown to involve a one-electron reduction. This is an agreement with the results obtained by Kaye and Stonehill[3]. This observation, is of great importance in relation to the free-radical mechanism of bacteriostasis by the aminoacridines put forward by Kaye[5]. With 5-aminoacridine the first step of the wave appears to correspond to a two electron reduction, in contradiction to Kaye's polarographically determined value of one electron. However the value of the initial current does not differ substantially from that observed with the remaining aminoacridines, and the different result is due to a longer half-time with 5-aminoacridine. It may therefore be the case that the higher result with this substance arises as a result of secondary processes, possibly of the nature discussed by Geske and Bard[9]. This possibility is being investigated.

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After Mr. Butler presented the paper there was a DISCUSSION.