THE CONTROLLED POTENTIAL REDUCTION OF CRYSTAL VIOLET AND BRILLIANT GREEN AT THE STIRRED MERCURY CATHODE

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The application of controlled potential coulometry to the electro-reduction of the triphenylmethane dyestuffs crystal violet and brilliant green is described below. The polarographic work by Kaye and Stone-hill (1952) indicated that, like the aminoacridines previously examined (Wilson, Butler, Ingle and Taylor, 1960), these were reduced in two stages each involving the uptake of one electron. The aim of the present work was to seek confirmation of this by direct measurement of the quantities of electricity involved in the reduction of a known amount of dyestuff.

Experimental Methods

The apparatus and techniques described by Wilson and others (1960) were employed. An electrolyte consisting of an aqueous buffer together with ethanol or dimethylformamide was depleted of reducible impurities by passing oxygen-free nitrogen through the solution and by prolonged electrolysis until the "background" current fell to a constant low value, usually not greater than 0·07 mA. To avoid changes in the composition of the mixed solvent, the gas was first passed through a wash bottle containing solvent of the same composition as that in the cell.

After adding a sample of the electro-reducible material, current-voltage diagrams were prepared using the method previously described. With ethanol as co-solvent, plots failed to show a sufficiently clear separation into the component steps to be of much value and 15 per cent dimethylformamide was used. Curves were then obtained showing two well defined steps but characterised by the presence of an anomalous fore-wave (Fig. 1a). Prolonged passing of nitrogen failed to remove this fore-wave but electrolysis for 20 min. at a potential of −700 mV was successful, and the subsequent current-voltage curve was found to be of normal appearance (Fig. 1b).

The appearance of the fore-wave was associated with the removal from the electrolysis cell of a few ml. of electrolyte in which the dye was dissolved before being returned to the cell. When this procedure was repeated omitting the dyestuff the fore-wave was still present in the buffer alone, and it is suggested that this phenomenon arises from the presence of some electro-reducible impurity in the dimethylformamide, reoxidised by contact with air. It had not been observed in parallel studies of aminoacridines in which ethanol was used as co-solvent.

The suggested procedure for coulometry in a solvent containing dimethylformamide is therefore as follows:

The mixed electrolyte (150 ml.) is submitted to electrolysis at the predetermined working potential in a stream of oxygen-free nitrogen until the background current had fallen to a low value. 5 ml. of the solution
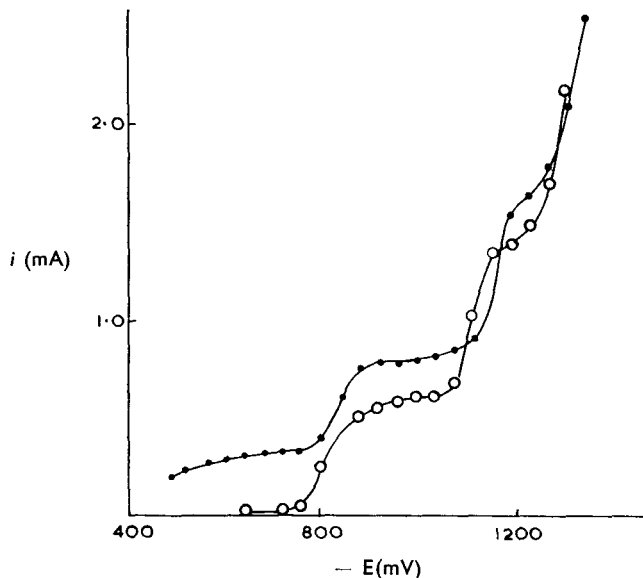

FIG. 1. Current - voltage curve of crystal violet in Sorensen's phosphate buffer (pH 7·4) (a, ●———● showing anomalous fore-wave; b, ○———○ after preliminary electrolysis at 700 mV) containing 15 per cent v/v of dimethylformamide.

is withdrawn, used to dissolve the weighed sample of dye and returned to the cell. Electrolysis is carried out under nitrogen for a further 20 min. at -700 mV to remove impurities. The potential is then adjusted to the appropriate value (-1000 mV for crystal violet), and decay of current with time observed. A typical current-time plot is shown in Fig. 2; from this a note was made of the initial current (I_0) and half-time ($t_{\frac{1}{2}}$) of the reaction. Substitution in the equation

$$Q = \frac{I_0 \times t_{\frac{1}{2}} \times 60}{0·693}$$ coulombs, where I_0 is in amperes and $t_{\frac{1}{2}}$ in min.,

gives the quantity of electricity which would be required for the reduction of the entire sample. The n value is calculated from

$$n = \frac{QM}{96·5\ W}$$

where M = molecular weight of the dye
W = wt. of sample in mg.

Experimental Results

Determination of the n value for the reduction of crystal violet at −1000 mV (corresponding to the first step of the polarographic wave) yielded the data in Table I.

TABLE I

N-VALUES FOR CRYSTAL VIOLET (FIRST STEP OF REDUCTION)

Wt. of sample mg.	I_0 mA	$t_{\frac{1}{2}}$ min.	n value
1·9	0·48	11·1	1·027
2·8	0·485	15·7	0·99
2·55	0·54	13·3	1·028
3·1	0·654	14·5	1·14
3·0	0·52	19·2	1·18
2·5	0·448	16·2	1·06
2·5	0·425	17·8	1·10
2·5	0·44	18·5	1·186

The n-value of 1 confirms the conclusions of Kaye and Stonehill (1952).

With brilliant green at slightly alkaline pH values the dyestuff was slowly converted from the coloured ionic form (I) to the colourless unionised and non-reducible form (II).
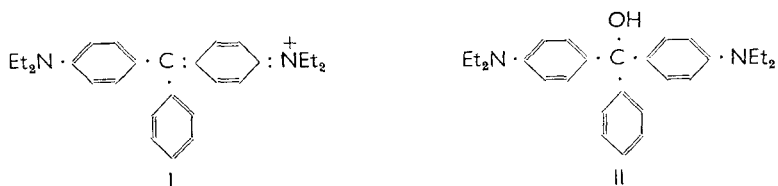
The colour of the solution slowly faded before electro-reduction could be commenced, and experimental n-values were in all cases less than 1 (Table II).

TABLE II

N-VALUES FOR BRILLIANT GREEN (FIRST STEP) pH 7·4*

Wt. of sample mg.	I_0 mA	$t_{\frac{1}{2}}$ min.	n value
2·1	0·30	9·7	0·63
1·7	0·355	9·2	0·83
1·95	0·38	6·8	0·57
3·0	0·325	11·7	0·55
3·0	0·35	31·8	0·62
3·0	0·185	23·1	0·61

When the reduction was investigated at pH 5·6* there was no noticeable fading of the colour of the solution prior to electrolysis, but separation of the current-voltage curve into two steps was insufficiently well marked to allow a satisfactory coulometric investigation of the first step. This is in accordance with Kaye and Stonehill (1952) who observed that the two steps of the polarographic wave were well separated only in alkaline solutions. Coulometric determinations were therefore carried out at a

* pH values of aqueous buffers used in the mixtures.

potential of $-1,200$ mV, corresponding to the top of the second wave; these indicated a n-value of 2.

It may be concluded that the triphenylmethane dyestuffs crystal violet and brilliant green are reduced at the stirred mercury cathode in two stages each involving one electron. The instability of brilliant green in alkaline solution has hindered a direct determination of the n-value corresponding to the first step, but the overall reduction (2 steps) has been shown to involve two electrons. In the case of crystal violet which is reduced in two steps of equal height, the first has been shown to correspond to the uptake of one electron.
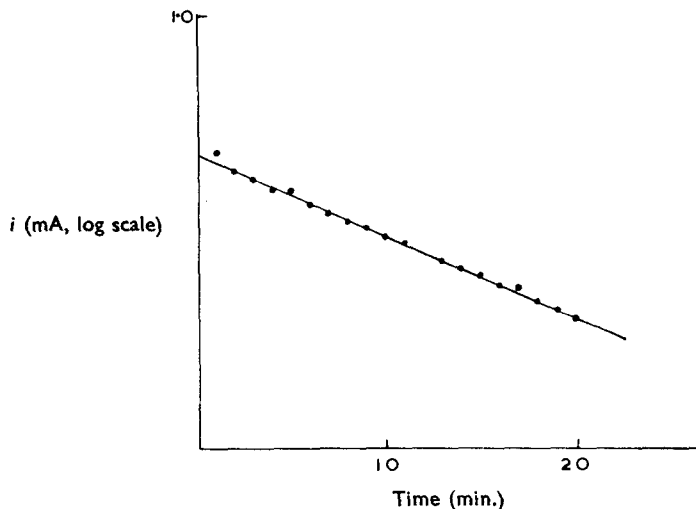

FIG. 2. Current decay plot for crystal violet (control potential — 1000 mV *vs* S.C.E.)

The conclusions reached by Kaye and Stonehill (1952) from polarographic data have thus been confirmed and may be of significance in view of Kaye's (1950) theory that bacteriostasis by certain dyes is related to free radical formation.

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