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The Reduction of Fluorescein in Aqueous Solution (at pH 6)

A New DISP2 Reaction

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It is shown using linear sweep and rotating-disc voltammetry that the reduction of the dye fluorescein at *ca.* pH 6 in aqueous solution proceeds *via* a DISP2 mechanism. In particular the rotating-disc measurements show voltammetric waves which display a shift in the half-wave potential with disc rotation speed and which also analyse to give curved Tafel plots entirely in agreement with the behaviour predicted theoretically for a DISP2 process on the basis of a reaction layer treatment.

A wide variety of electrode reactions, particularly those of organic molecules, involve the transfer of two electrons. Such reactions were first interpreted in terms of the familiar ECE mechanism,[1, 2] in which the first electron transfer (E) is followed by a chemical step (C), the product of which readily undergoes a further electron transfer (E) at the potentials concerned. It is now very well established, however, that within the broad framework of the ECE scheme other reaction pathways may operate.[3–7] In particular, the second electron transfer may occur homogeneously *via* a disproportionation reaction, rather than heterogeneously at the electrode surface. The overall picture may be described by the following general kinetic scheme:

$$A + e \rightleftharpoons B \quad (1)$$

$$B \rightleftharpoons C \quad (2)$$

$$C + e \rightleftharpoons D \quad (3)$$

$$B + C \rightleftharpoons A + D \quad (3')$$

Three limiting cases have been delineated by Savéant[6, 7] as arising out of steps (1)–(3′) and these are summarised in table 1, from which it can be seen that those mechanisms involving a homogeneous electron transfer are labelled DISP. A numerical suffix (1 or 2) is added to show the order of the rate-determining step.

In this paper we are concerned with the DISP2 process. This mechanism has been treated theoretically for quiescent solutions in respect of potential-step chronoamperometry,[8–10] chronopotentiometry,[11] linear sweep and cyclic voltammetry[5, 7, 12] and convolution potential sweep voltammetry.[13] We have elsewhere[14] considered theory for this reaction at the rotating disc electrode under the condition that the following chemical reaction is sufficiently fast that we could employ the reaction layer approximation. It was found that the current–voltage curves predicted, produced pronouncedly curved Tafel plots when analysed in the usual way allowing for the effect of mass transport.[15] It was also predicted that the half-wave potential shifted with the electrode rotation speed by (*ca.*) 20 mV per decade (at 25 °C).

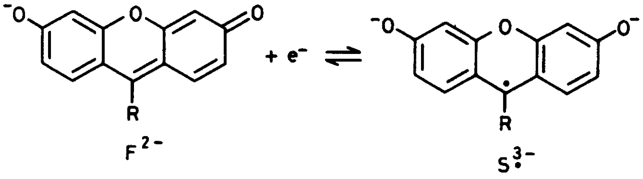
The purpose of this paper is to verify experimentally the above predictions in relation to the DISP2 reaction at the rotating-disc electrode. The number of systems reported in the literature as showing DISP2 behaviour is rather limited, and includes the reduction of carbonyl compounds in alkaline ethanol[12] and imines in DMF and acetonitrile,[16] as

Table 1. The ECE–DISP mechanisms

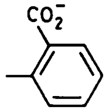
	step			
mechanism	1	2	3	3′
ECE		r.d.s.[a]	—	X[b]
DISP1		r.d.s.	X	—
DISP2		—	X	r.d.s.

[a] r.d.s. = rate-determining step in the mechanism. [b] X = unimportant in the mechanistic scheme.

well as the exhaustively studied reduction of the uranyl ion, UO^{2+}.[17] In this work we report a new DISP2 system, namely the reduction of the dye fluorescein in aqueous solution at *ca.* pH 6. This system has previously been studied at very high pH (> 12) where a one-electron reduction to the stable semi-fluorescein radical is observed:[18-20]



where — R =



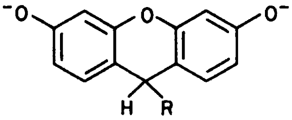
On lowering the pH to the region 9–10 it has been shown[21-23] using a combination of *in situ* electrochemical e.s.r. and channel electrode measurements that a two-electron process occurs forming leuco-fluorescein *via* a DISP1 mechanism:

electrode $F^{2-} + e^- \rightleftharpoons S^{\cdot 3-}$

solution $S^{\cdot 3-} + H^+ \rightarrow SH^{\cdot 2-}$

$SH^{\cdot 2-} + S^{\cdot 3-} \rightleftharpoons F^{2-} + L^{3-}$

where $L^{3-} =$



This assignment of the DISP1 mechanism was subsequently confirmed by Harland[24] using rotating-disc voltammetry. The behaviour at pH 6 is shown here to again involve the formation of leucofluorescein *via* a two-electron process, except that, because of the higher proton levels, the rate-determining step is no longer the protonation of semi-fluorescein but the disproportionation reaction between protonated semi-fluorescein and semi-fluorescein *i.e.* a DISP2 reaction.

Experimental

Rotating-disc and Linear Sweep Voltammetry

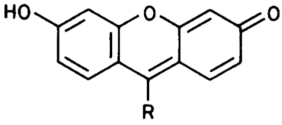
An Oxford Electrodes (Oxford) rotating-disc assembly, motor controller and potentiostat were used together with a carefully polished (0.25 μm diamond grit) copper rotating-disc electrode of radius 3.565 mm. The latter was plated with triply distilled mercury using a procedure described previously.[25] The mercury surface was renewed between each voltammetric measurement. In the case of the linear sweep measurements, the rotating disc was replaced with electrodes formed by sealing copper wires of radius 0.85 mm, into glass tubing of similar bore, and grinding the ends flat before polishing and mercury plating them (as above). Sweep rates in the range 50–2000 mV s^{-1} were employed in the linear-sweep experiments and the resulting current–voltage curves were recorded using a Gould digital storage oscilloscope (model 054000).

Solutions and Procedure

Solutions were made up using doubly distilled deionised water (resistivity $> 10^7$ Ω cm) and were thoroughly purged of oxygen by passage of a stream of pre-purified nitrogen gas through them before use. In all work the solutions were maintained at a temperature to within 0.1 °C of 25 °C. Potentials were recorded against a saturated calomel electrode. Buffered solutions in the pH range 5.8–6.0 were obtained using potassium dihydrogen phosphate–sodium hydroxide buffers to which potassium chloride was added both to serve as a background electrolyte and to maintain a constant ionic strength of 1 mol dm^{-3}. AnalaR grade reagents were used for these solutions. pH measurements were made using a Radiometer PHM 64 meter. Fluorescein was the best grade available (obtained from Fluka).

Results and Discussion

Experiments were carried out on the reduction of the dye fluorescein at pH 5.88 in the concentration range 3×10^{-4} to 5×10^{-4} mol dm^{-3}, the upper limit corresponding to the maximum solubility of the material at this pH. U.v.–visible spectra of these solutions coupled with the observations of Lindquist[26] enabled the deduction that, at this pH, the dye exists as the mono-anion (FH^-):



Under the above conditions, voltammetric waves at the rotating disc were found to obey the Levich equation:[27]

$$I_{LIM} = 1.554nFAD^{\frac{2}{3}}v^{-\frac{1}{6}}W^{\frac{1}{2}}[FH^-] \qquad (4)$$

where I_{LIM} is the diffusion-limited current, n is the number of electrons transferred in the reduction, F is Faraday's constant, D is the diffusion coefficient of FH^-, v is the kinematic viscosity, and W is the disc rotation speed (Hz). Our data shown in fig. 1 reveal the reduction to be a two-electron process at all the rotation speeds examined, and that the value of D is $3.2 \pm 0.1 \times 10^{-6}$ cm^2 s^{-1}. This is very close to the value found at higher pH for F^{2-}.[18-20]

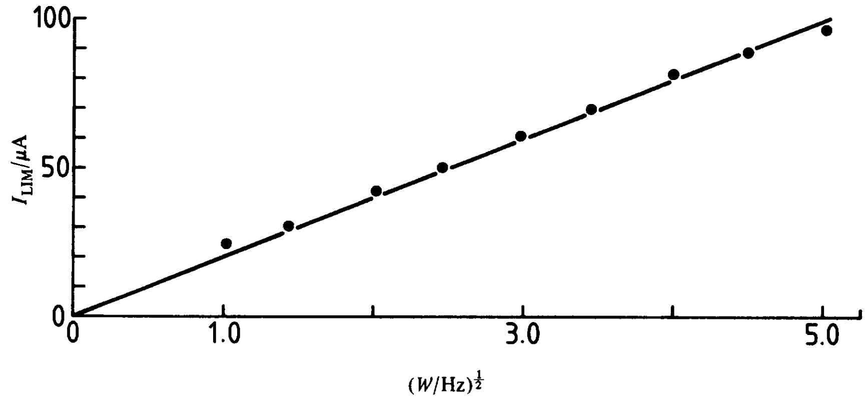


Fig. 1. Analysis of rotating disc voltammograms for the reduction of fluorescein at pH 5.88 shows two-electron Levich behaviour.

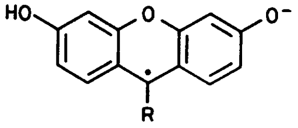
For the reasons developed below, we suggest that the reduction proceeds *via* a DISP2 mechanism as follows:

electrode $\mathrm{FH^- + e^- \rightleftharpoons SH^{\bullet 2-}}$

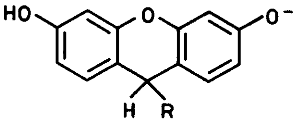
solution $\mathrm{SH^{\bullet 2-} + H^+ \rightleftharpoons SH_2^{\bullet -}}$

$$\mathrm{SH^{\bullet 2-} + SH_2^{\bullet -} \xrightarrow{k} FH^- + LH^{2-}}$$

where $\mathrm{SH^{\bullet 2-}}$ =



and $\mathrm{LH^{2-}}$ =



Since $\mathrm{LH^{2-}}$ is known to be the product of the reduction[23] the reaction must involve two-electron transfers and one chemical step (protonation). On the basis of our previous studies, it is thus reasonable to assume that the mechanism is likely to be ECE, DISP1 or DISP2, and we now attempt to distinguish between these possibilities.

That the process is accurately two-electron at all practical rotation speeds implies a fast following chemical reaction (close to diffusion-controlled for a DISP2 process and greater than *ca.* 10^3 $\mathrm{s^{-1}}$ for an ECE or DISP1 reaction[28] and precludes mechanistic analysis *via* the limiting current–rotation speed behaviour. However, we have shown that ECE, DISP1 and DISP2 processes are discernable from one another through a combination of Tafel analysis and the half-wave potential-rotation speed behaviour.[14, 24] Table 2 summarises the characteristic responses of the three mechanisms under these analyses. The distinction between DISP2 and the other mechanisms is evident. The discrimination between ECE and DISP1 from the $E_{\frac{1}{2}}$-rotation speed data is possible either (*a*) by determining the absolute magnitude of the shift from the standard electrode

Table 2. ECE, DISP1 and DISP2 behaviour at the rotating-disc electrode

	Tafel analysis		$E_{\frac{1}{2}}-W$ analysis
mechanism	shape	$(\mathrm{d}E/\mathrm{d}\log[I^{-1}-I_{\mathrm{LIM}}^{-1}])/$ mV per decade	$(\mathrm{d}E_{\frac{1}{2}}/\mathrm{d}\log W/\mathrm{Hz})/$ mV per decade
ECE	linear	60	30
DISP1	linear	60	30
DISP2	curved	40–60	20

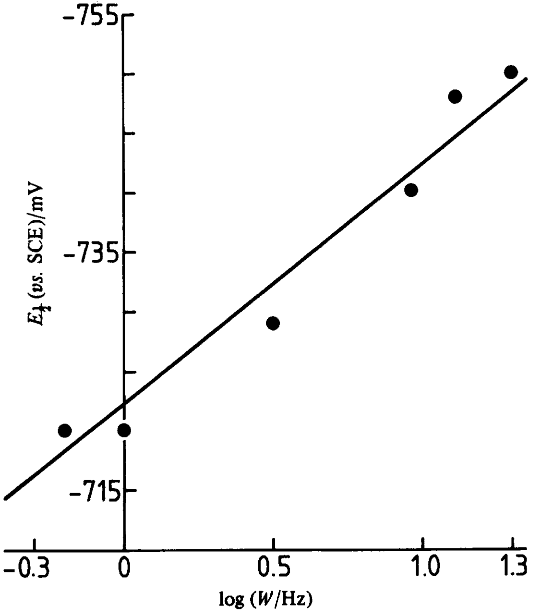


Fig. 2. Variation of the half-wave potential with rotation speed for the same voltammograms as analysed in fig. 1. The straight line represents the theoretically predicted behaviour.[14]

potential of the redox couple when the latter is measured under conditions free from kinetic complications (*e.g.* at rotation speeds where the following kinetics can be 'outrun'), or (*b*) through independent knowledge of the first-order rate constant describing the chemical step (*e.g.* from spectroelectrochemistry).

Fig. 2 shows the experimentally-determined variation of half-wave potential with rotation speed for the reduction of fluorescein. The line drawn has the slope of 20 mV predicted for a DISP2 reduction. Moreover, the potential range in which the half-wave potentials lie as compared to the standard potential for the reduction of FH^- to $SH^{\cdot 2-}$ (which may be estimated given the pK_a values of FH^- and $SH^{\cdot 2-}$,[26] and the electrode potential of the reversible reduction of unprotonated fluorescein to semi-fluorescein[24]) indicates that the disproportionation reaction between $SH^{\cdot 2-}$ and $SH_2^{\cdot -}$ is close to, or actually is diffusion-controlled (*ca.* 10^{10} $\mathrm{dm^3\ mol^{-1}\ s^{-1}}$).

Further evidence that the reduction process is a DISP2 reaction comes from analysis of the shapes of the voltammetric waves. Fig. 3 shows a conventional mass-transport-corrected Tafel plot [of E against log $(I^{-1}-I_{\mathrm{LIM}}^{-1})$] for a wave run at 9.25 Hz. The plot is significantly curved as has been predicted for DISP2 reactions.[14] Also shown is a curve calculated theoretically, as described elsewhere, for a rate constant $k = 5\times10^9$ $\mathrm{dm^3}$

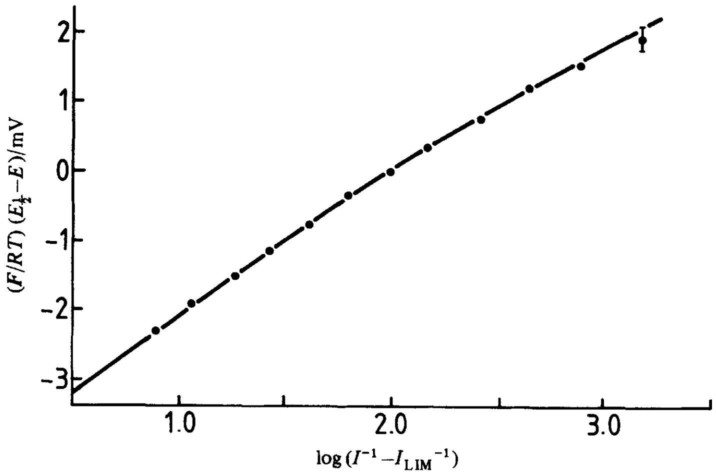


Fig. 3. Analysis by mass-transport-corrected Tafel plot of a voltammogram recorded at 9.25 Hz. The curve drawn through the points represents the theoretically predicted DISP2 behaviour.[14]

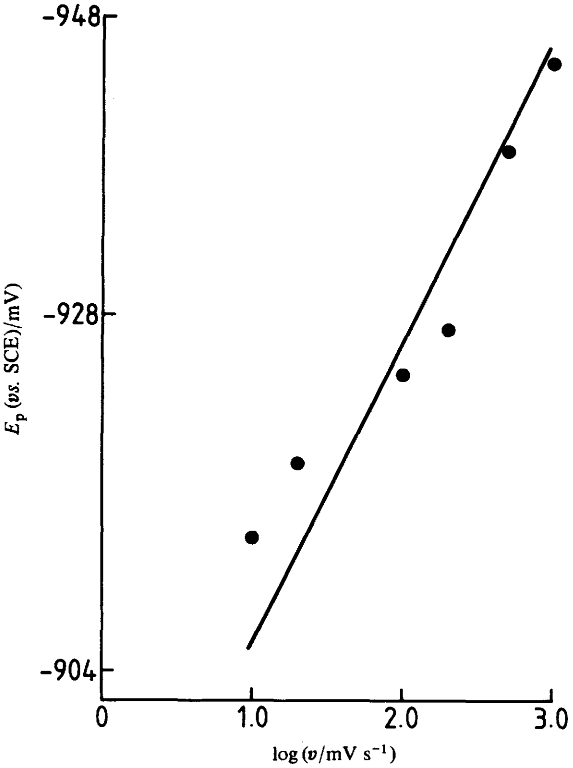


Fig. 4. The dependence on scan rate, v, of the peak potential, $E_{\rm p}$, in the linear sweep voltammogram of fluorescein reduction at pH 5.88. The straight line represents the theoretically predicted behaviour.[7]

mol^{-1} s^{-1}. An excellent match is seen and the agreement with the rate constant estimated from the shift in half-wave potential is satisfactory.

As a final check that the reaction is indeed DISP2 we have carried out linear sweep voltammetry (LSV) on the fluorescein system. Savéant and co-workers[7] have demonstrated that the shift in peak potentials with scan rate [dE_p/dlog (scan rate)] is different for DISP2 systems (20 mV per decade) and ECE–DISP1 systems (30 mV per decade). Fig. 4 shows our LSV data and the line drawn through the points has the slope of 20 mV. This may be taken as confirmation that the mechanism is indeed a DISP2 process.

In conclusion we can say that the reduction of fluorescein at pH 6 has been characterised as a new DISP2 process. Furthermore, we have experimentally established a rotating-disc method for the discrimination of ECE–DISP1 and DISP2 reactions. Hence together with our earlier work[24] on the ECE *vs*. DISP1 problem it is now possible to distinguish between all three mechanistic pathways using the rotating-disc electrode.

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References

1 A. C. Testa and W. H. Reinmuth, *Anal. Chem.*, 1961, **33**, 320.
2 A. C. Testa and W. H. Reinmuth, *J. Am. Chem. Soc.*, 1961, **83**, 784.
3 M. D. Hawley and S. W. Feldberg, *J. Phys. Chem.*, 1966, **70**, 3459.
4 M. D. Hawley and S. W. Feldberg, *J. Phys. Chem.*, 1967, **71**, 851.
5 M. Mastragostino, L. Nadjo and J. M. Savéant, *Electrochim. Acta*, 1968, **13**, 721.
6 C. Amatore and J. M. Savéant, *J. Electroanal. Chem.*, 1977, **85**, 27.
7 C. Amatore, M. Gareil and J. M. Savéant, *J. Electroanal. Chem.*, 1983, **147**, 1.
8 G. L. Booman and D. T. Pence, *Anal. Chem.*, 1965, **37**, 1367.
9 S. W. Feldberg, *J. Phys. Chem.*, 1969, **73**, 1238.
10 C. Amatore and J. M. Savéant, *J. Electroanal. Chem.*, 1979, **102**, 21.
11 S. W. Feldberg and C. Auerbach, *Anal. Chem.*, 1964, **36**, 505.
12 L. Nadjo and J. M. Savéant, *J. Electroanal. Chem.*, 1971, **33**, 419.
13 J. C. Imbeaux and J. M. Savéant, *J. Electroanal. Chem.*, 1973, **44**, 169.
14 R. G. Compton, D. Mason and P. R. Unwin, preceding paper.
15 W. J. Albery, *Electrode Kinetics* (Oxford University Press, Oxford, 1975), p. 79.
16 C. P. Andrieux and J. M. Savéant, *J. Electroanal. Chem.*, 1971, **33**, 453.
17 M. Mastragostino and J. M. Savéant, *Electrochim. Acta*, 1968, **13**, 751, and references therein.
18 B. A. Coles and R. G. Compton, *J. Electroanal. Chem.*, 1983, **144**, 57.
19 R. G. Compton, D. J. Page and G. R. Sealy, *J. Electroanal. Chem.*, 1984, **163**, 65.
20 R. G. Compton and A. M. Waller, *J. Electroanal. Chem.*, 1985, **195**, 289.
21 R. G. Compton, P. J. Daly, P. R. Unwin and A. M. Waller, *J. Electroanal. Chem.*, 1985, **191**, 15.
22 R. G. Compton and P. R. Unwin, *J. Electroanal. Chem.*, 1986, **205**, 1.
23 R. G. Compton and A. R. Hillman, *Chem. Br.*, 1986, **22**, 1088.
24 R. G. Compton, R. G. Harland, P. R. Unwin and A. M. Waller, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 1261.
25 P. J. Daly, D. J. Page and R. G. Compton, *Anal. Chem.*, 1983, **55**, 1191.
26 L. Lindquist, *Arkiv. Kemi*, 1960, **16**, 79.
27 V. G. Levich, *Physicochemical Hydrodynamics* (Prentice–Hall, Englewood Cliffs, NJ, 1962).
28 L. S. Marcoux, R. N. Adams and S. W. Feldberg, *J. Phys. Chem.*, 1969, **73**, 2611.

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