Voltammetric Behaviour of 2-Amino-5-methyl-1,3,4-thiadiazole at a Pyrolytic Graphite Electrode

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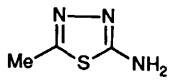
Anodic oxidation of 2-amino-5-methyl-1,3,4-thiadiazole **1** was studied at a pyrolytic graphite electrode, over a wide pH range 2.23–10.7 in phosphate buffers, by various electrochemical and spectroscopic techniques. Compound **1** undergoes a $2e^-$, $2H^+$ oxidation process to give an electroactive azo product which has been characterised, and a suitable mechanism for the electro-oxidation process is suggested.

Aminothiadiazole derivatives have attracted considerable attention in the last few years due to their applications in the treatment of various diseases. 2-Amino-5-substituted 1,3,4-thiadiazoles have been found to prolong significantly the life-span of tumour-bearing mice.[1] The activity of aminothiadiazoles have also been claimed to be much superior to mitomycin C agent P-388 against murine leukaemia.[2] As electrochemical studies provide a unique and invaluable insight into the redox behaviour of biologically important compounds, the polarographic behaviour of several thiadiazoles has also been reported in the literature.[3,4] Nevertheless, most of the investigations reported deal with the reduction of these compounds at a dropping-mercury electrode (DME) and nothing significant is known about their electro-oxidation behaviour. In view of the importance of oxidation reactions in biological systems,[5] it was considered interesting to elucidate the redox behaviour of a simple thiadiazole, *viz.* 2-amino-5-methyl-1,3,4-thiadiazole **1**. The present paper summarises the results of our study on the voltammetric redox behaviour of compound **1** at a pyrolytic graphite electrode with special emphasis on the elucidation of the redox mechanism.



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Experimental

2-Amino-5-methyl-1,3,4-thiadiazole was obtained from Sigma and was used as received. The azo product was synthesized in the laboratory by the method reported in the literature.[6] The purity of the synthesized compound was ascertained by TLC, m.p., and repeated recrystallisation. A pyrolytic graphite electrode (area 0.04 cm^2) was prepared by the method reported in the literature.[7] The equipment used for voltammetry, coulometry, spectral studies, and controlled-potential electrolysis were essentially the same as has been reported earlier.[8] UV spectra were recorded on Hitachi videodisplay spectrophotometer, and the mass spectrum of the product was reported on a JEOL JMS D300 mass spectrophotometer. All potentials were referred to saturated calomel electrode (SCE) at 25 °C.

Stock solution (10 mmol dm^{-3}) of 2-amino-5-methyl-1,3,4-thiadiazole was prepared in doubly distilled water. Working solutions were prepared by mixing an aliquot (1 cm^3) of the stock solutions with phosphate buffers[9] (9 cm^3) of appropriate pH. Purified nitrogen gas was bubbled through the solution for 8–10 min and the voltammograms were then recorded. Controlled-potential electrolysis (CPE) of compound **1** was carried out in an H-type cell using a pyrolytic graphite plate (6 × 1 cm^2) as working, SCE as reference, and a platinum gauze cylinder as an auxiliary electrode, respectively. The progress of electrolysis was monitored by recording cyclic voltammograms at different time intervals. When the oxidation peak completely disappeared, electrolysis was stopped and the electrolysed solution was transferred from the cell and was lyophilised. The concentration slurry exhibited only one spot on TLC (R_f ~0.8, benzene–MeOH 3:7), indicating the formation of one major product during the electro-oxidation of compound **1**. The slurry was then extracted with 1,4-dioxane (BDH) (5 cm^3) to remove the buffer constituents. The dioxane extract was concentrated under reduced pressure and the light yellow product obtained was analysed by its m.p., and IR, NMR and mass spectra.

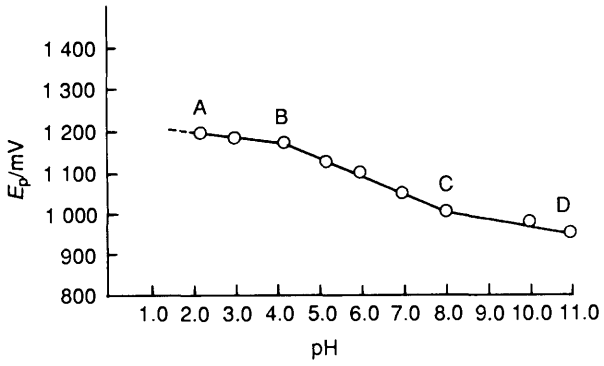


Fig. 1. Observed dependence of peak potential E_p for peak 1_a on the pH for 1.0 mmol dm^{-3} 2-amino-5-methyl-1,3,4-thiadiazole at PGE. Scan rate 10 mV s^{-1}.

Results and Discussion

Linear Sweep and Cyclic Voltammetry.—Linear sweep voltammograms (10 mV s^{-1}) of compound **1** in phosphate buffers in the pH range 2.2–10.7 show only one well defined oxidation peak. The peak potential of this peak was pH dependent and shifted towards the less positive potential on an increase in pH. The plot of E_p *vs.* pH (Fig. 1) indicates two breaks at pH ~4.2 and ~8.0. The dependence of E_p on pH can be represented by eqns. (1)–(3).

$$E_p \text{ (pH 2.2–4.2)} = [1.225 - 0.015\,\text{pH}]\ \text{V} \quad (1)$$

$$E_p \text{ (pH 4.2–8.0)} = [1.360 - 0.046\,\text{pH}]\ \text{V} \quad (2)$$

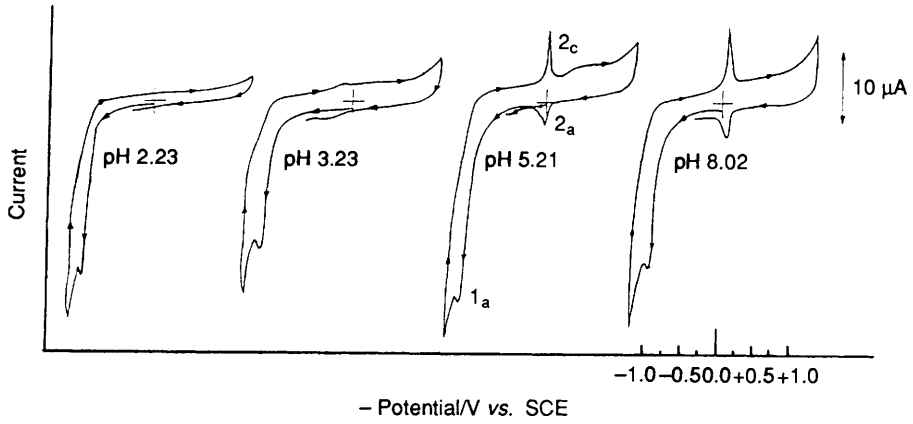


Fig. 2. Cyclic voltammograms recorded for 1.0 mmol dm^{-3} 2-amino-5-methyl-1,3,4-thiadiazole at PGE in phosphate buffers of different pH. Sweep rate 50 mV s^{-1}.

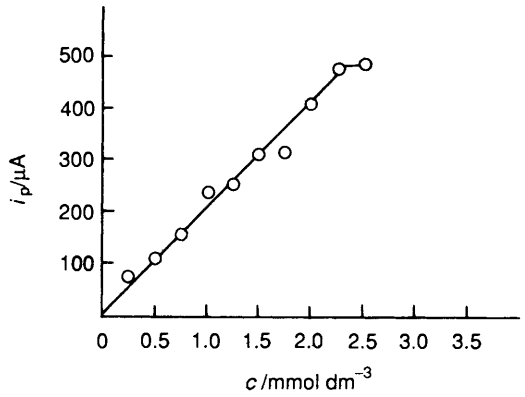


Fig. 3. Plot of peak current (i_p) *versus* concentration of 2-amino-5-methyl-1,3,4-thiadiazole for peak 1_a at pH 3.8.

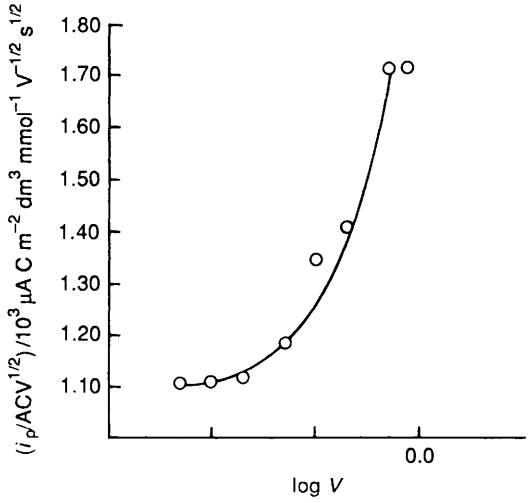


Fig. 4. Plot of peak current function ($i_p/ACV^{\frac{1}{2}}$) *versus* log V of 2-amino-5-methyl-1,3,4-thiadiazole at pH 3.8.

$$E_p \text{ (pH 8.0–10.7)} = [1.160 - 0.018 \text{ pH}] \text{ V} \quad (3)$$

At pH <3.2, a 1 mmol dm^{-3} solution of compound **1** exhibits only one oxidation peak (1_a) when the sweep was initiated in the positive direction. No other peak was observed in the reverse sweep, whereas at pH > 3.2, a well defined reduction peak (2_c) was observed on the reverse sweep, which formed a quasireversible couple with peak 2_a observed on the subsequent sweep towards positive potential. Some representative CV graphs of compound **1** at a sweep rate of 50 mV s^{-1} are presented in Fig. 2.

The peak current for the oxidation peak 1_a increased with an increase in concentration of compound **1** up to *ca.* 2.25 mmol dm^{-3} and became more or less constant thereafter (Fig. 3). The peak current function ($i_p/ACV^{\frac{1}{2}}$) for peak 1_a increased rapidly with increasing sweep rate and a plot of $i_p/ACV^{\frac{1}{2}}$ *versus* log V is presented in Fig. 4. This behaviour clearly shows the adsorption phenomenon of the reactant at the pyrolytic graphite electrode surface prior to scanning,[10] indicating thereby that under the voltammetric conditions employed the oxidation of compound **1** is an overall irreversible process.[11]

The peaks of the redox couple $2_c/2_a$, observed after scanning through oxidation peak 1_a, are highly symmetrical. The quotient of peaks $2_a/2_c$ was always found to be constant (0.5). In phosphate buffers (μ 0.5) the E_p-values for peak 2_a and 2_c are pH dependent according to eqns. (4) and (5).

$$E_p \text{ (pH 3.23–1.94)} = [0.205 - 0.032 \text{ pH}] \text{ (for peak } 2_c) \quad (4)$$

$$E_p \text{ (pH 4.21–9.0)} = [0.151 - 0.025 \text{ pH}] \text{ (for peak } 2_a) \quad (5)$$

Controlled-potential Electrolysis and Coulometry.—Controlled-potential electro-oxidation of compound **1** at potentials more positive than peak 1_a was carried out at pH 3.2 and 7.8, and the progress of electrolysis was monitored by recording of the cyclic voltammograms at different time intervals. As the electrolysis progressed the solution turned light yellow in colour and the surface of the electrode was coated with light yellow material. Peak 1_a systematically decreased, whereas peaks 2_c and 2_a appeared in the cyclic voltammogram when the direction of initial sweep was negative, indicating thereby that the species responsible for peak 2_c is present in the solution. The height of peaks 2_c and 2_a did not change significantly during the course of oxidation, probably because of adsorption of species responsible for peak 2_c at the electrode surface. Some of the cyclic voltammograms observed during electro-oxidation of compound **1** at pH 7.8 are presented in Fig. 5 (*a*, *b*, *c* and *d*). The appearance of peak $2_c/2_a$ in the exhaustively electrolysed solution of compound **1**, Fig. 5(*d*), clearly indicates that the product of electro-oxidation of compound **1** is electroactive in nature.

The value of n, number of electrons involved in the electro-oxidation, was determined by using a conventional coulometer in series and the value of n was found to be 2.0 ± 0.1 (average ± maximum deviation) over the pH range 2.2–10.7.

Spectral Studies.—The UV spectra of 2-amino-5-methyl-1,3,4-thiadiazole was recorded at different pH-values to

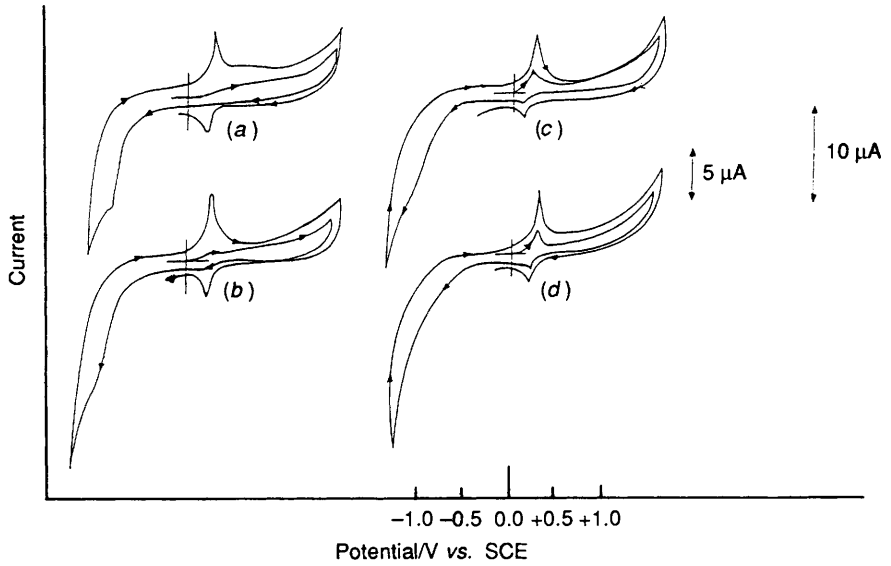


Fig. 5. Observed cyclic changes during electro-oxidation of 2-amino-5-methyl-1,3,4-thiadiazole (1.0 mmol dm^{-3}) at pH 7.8. Potential applied 1.2 V. Curves were recorded after 0 (*a*); 20 (*b*); 50 (*c*) min of electrolysis, and (*d*) after exhaustive electrolysis.

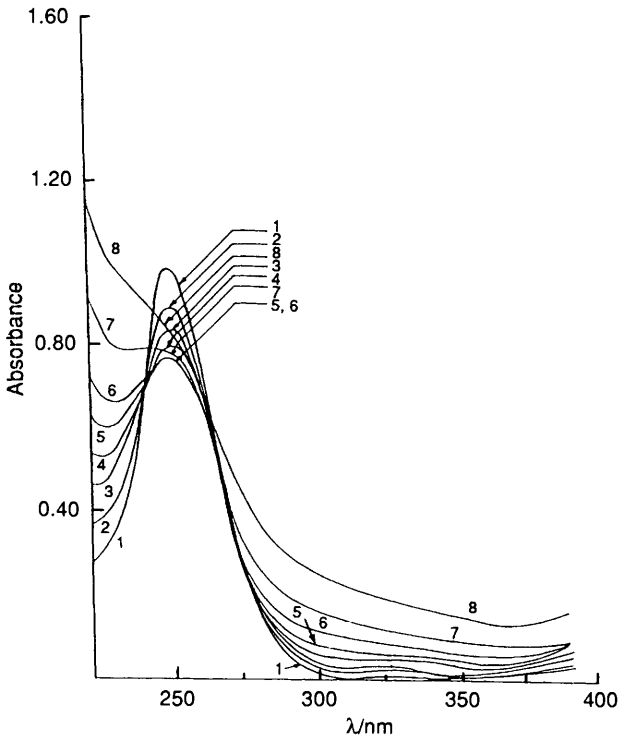
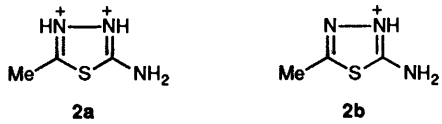


Fig. 6. Spectral changes for 0.2 mmol dm^{-3} 2-amino-5-methyl-1,3,4-thiadiazole at pH 3.88. Curves 1–6 were recorded at an interval of 5 min and curves 7 and 8 were recorded after 40 and 50 min of electrolysis, respectively.



2a **2b**

determine its pK_a. In the entire pH-range 2.2–10.7 only one UV peak, with λ_{max} ~250 nm, was observed. However, the plot of absorbance against pH exhibited two inflections, at pH *ca.* 4.2 and *ca.* 7.8, and were similar to the E_p *vs.* pH breaks. It seems reasonable to conclude that at pH <4.2, compound **1** exists as a protonated species. As protonation in the *N*-heterocyclic amino compound occurs first in the ring nitrogen, the species at pH <4.2 will be **2a** and in the pH range 4.2–8.0 will most probably be the monoprotonated molecule **2b**.

The progress of electrolysis of compound **1** (0.2 mmol dm^{-3}) was also monitored at pH 3.2 and 7.8 by recording of the UV spectral changes to detect the formation of any UV-absorbing intermediate. At pH 3.2 compound **1** exhibits a well defined UV band at 250 nm. With the progress of electrolysis the absorbance at λ_{max} decreased whereas absorbance in the wavelength region 275–400 nm systematically increased (curves 1–6, Fig. 6). After 40 min the absorbance at λ_{max} increased again (curves 7, 8) and became constant. The increase in absorbance in the longer wavelength region clearly indicated that the product of electro-oxidation of compound **1** possessed more extensive π-conjugation than did the starting material. Identical behaviour was observed at pH 7.8.

Product Characterisation.—The product of electro-oxidation of compound **1** formed at pH 3.2 and 7.8 was characterised. The light yellow material obtained from the dioxane extract (see the Experimental section) exhibited a strong band at 1690 cm^{-1} in IR spectrum, indicating the possibility of an azo group in the product. The NMR spectrum of compound **1** exhibited a strong signal at δ_H 6.67, corresponding to the presence of an amino group, whereas this signal did not appear in the NMR spectrum of the product of electro-oxidation. This clearly indicated that oxidation in compound **1** occurred at the amino group. The azo structure for the product was also confirmed by the mass spectrum. A clear molecular ion peak at *m/z* 226 confirmed the azo structure. The high mass peaks observed in the fragmentation pattern were at 226 (20.1%), 225 (8.6), 221 (6.0), 201 (9.8), 157 (10.1), 156 (6.1), 142 (7.3), 115 (30.1), 113 (22.6), 112 (11.8) and 111 (2.8).

To provide further confirmation of the structure of the product, cyclic voltammograms in phosphate buffers were recorded by initiating the sweep in the negative direction. The presence of peaks 2_c and reverse sweep 2_a at potentials similar to that observed for authentic azo compound and the couple $2_c/2_a$ and 2-amino-5-methyl-1,3,4-thiadiazole clearly indicated that the product of electro-oxidation is 2,2′-azobis-(5-methyl-1,3,4-thiadiazole). A comparison of cyclic voltammograms of compound **1**, authentic azo compound and product is presented in Fig. 7.

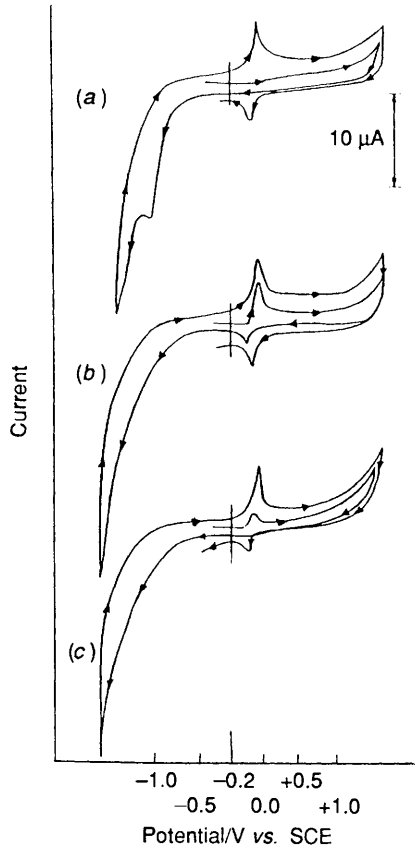


Fig. 7. Recorded cyclic voltammograms of 2-amino-5-methyl-1,3,4-thiadiazole (*a*), authentic azo compound (*b*), and electro-oxidised product of 2-amino-5-methyl-1,3,4-thiadiazole (*c*) at PGE in phosphate buffer pH 7.8. CV were recorded with a shift of 200 mV in the peak potential.

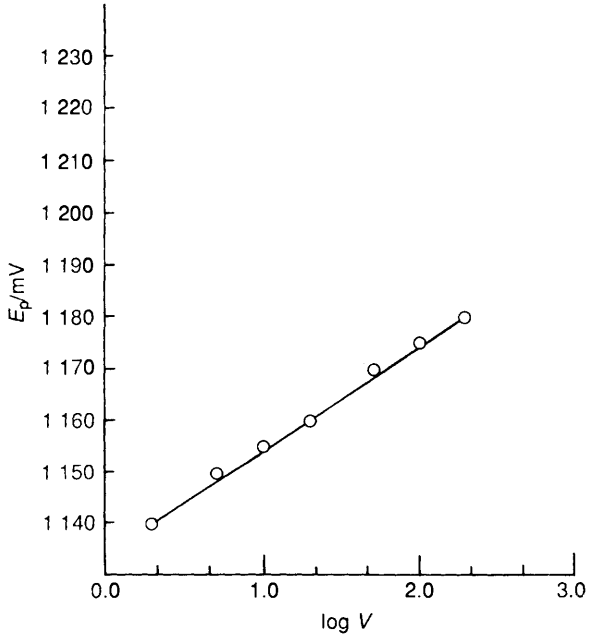
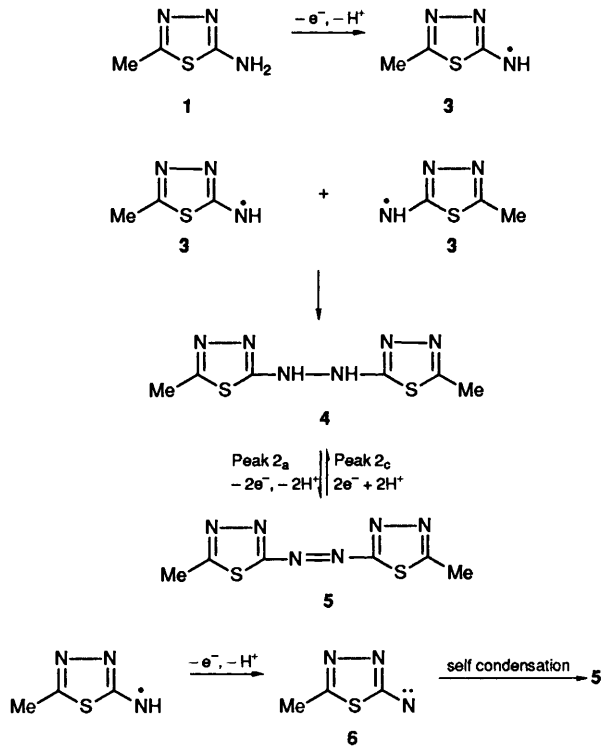


Fig. 8. Plot of E_p *vs.* log v for 1_a for 2-amino-5-methyl-1,3,4-thiadiazole at PGE in phosphate buffer pH 3.88.

Reaction Scheme.—The experimental evidence presented here clearly indicated that compound **1** is oxidised in a $2e^-$, $2H^+$ reaction to give an azo compound as the product of electro-oxidation. The following tentative reaction scheme can be suggested for the formation of the azo product. The first step of the reaction for the oxidation of compound **1** should be a $1e^-$, $1H^+$ oxidation to give a free radical **3**, as reported in the literature for a large number of heterocyclic amino compounds.[12] Species **3** can undergo deactivation by two major routes. In the first route (Scheme 1) a radical–radical coupling can lead to a hydrazo molecule **4**, which is more easily oxidisable than is starting compound **1** and hence its $2e^-$, $2H^+$ oxidation would give an azo species **5**. As azo compounds are easily reducible over a wide pH range,[13,14] reduction of azo species **5** to the hydrazine **4** and oxidation of compound **4** to the azo species **5** is clearly observed in reactions which give rise to peaks $2_c/2_a$.



Scheme 1. Tentative mechanism suggested for the electro-oxidation of 2-amino-5-methyl-1,3,4-thiadiazole.

The second route of deactivation involves a further $2e^-$, $2H^+$ oxidation of the radical of **3** to give a nitrene species **6**, which on self condensation[15] leads to the formation of an azo molecule (**5**). A redox couple $2_c/2_a$ similar to that of the first route is also expected in this reaction scheme. To distinguish between the two possible courses of electro-oxidation the method of Nadjo *et al.*[16,17] was used. The variation of E_p for the oxidation peak 1_a was monitored against the increase in sweep rate and the plot of E_p *versus* log *V* (Fig. 8) indicate a slope of 20 mV, confirming the radical–radical coupling in the present studies and hence it is concluded that the formation of an azo product occurs *via* the radical–radical coupling mechanism.

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