Aust. J. Chem., 1983, **36**, 507–16

Some Electrochemical and Photoelectrochemical Properties of 3-Amino-7-dimethylamino-2-methylphenazine (Neutral Red) in Aqueous Solution

Christopher S. Halliday[A,B] *and Dennis B. Matthews*[A]

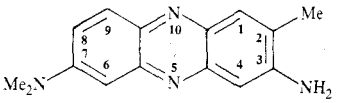
[A] School of Physical Sciences, The Flinders University of South Australia, Bedford Park, S.A. 5042.
[B] Author to whom correspondence should be addressed.

Abstract

Neutral Red has been shown to undergo electrochemical and photoelectrochemical reactions in aqueous solution. At a pH above the pK_a, the dye was oxidized with the loss of one proton with each electron. Similarly the protonated dye was oxidized at pH values below the pK_a. Over the pH range examined, 1·0–9·2, Neutral Red was reduced with one proton being gained with each electron. The neutral radical which was formed could be further reduced. When the pH was above the pK_a the dye was reduced by an H e e H mechanism, and when the pH was below the pK_a the reduction proceeded by an H e H e mechanism with the possibility of further protonation.

On a platinum electrode, Neutral Red underwent a photoelectrochemical oxidation at pH 7·2. The voltammogram showed a broad peak centred at 0 V (s.c.e.).

Neutral Red, viz. 3-amino-7-dimethylamino-2-methylphenazine, has long been used as a pH indicator in biological studies[1] because its red–yellow colour change at pK_a 6·7[2] falls in the pH range close to that of biological media. However, problems were encountered in studies of photosynthetic reactions when Neutral Red was used as a spectroscopic probe of the internal pH of thylakoids.[3] The observed changes in the probe's absorbance were consistent with acidification of the medium but were much larger than expected. One explanation is that the dye was protonated preferentially in the aqueous phase; this indicated a variation of activity with location.[3]



Neutral Red

Other explanations for these results are that the unexpected decrease in the absorbance was due to Neutral Red acting as an artificial electron donor or acceptor in the photosynthetic reactions, and that the decrease was due to photoelectrochemical reactions of Neutral Red. As the dye absorbs light, it may undergo a charge-transfer reaction with species generated in the photosynthetic reactions.

In concentrated acid solutions Havemann *et al.*[4] examined solutions of Sn^{2+} ions and Neutral Red at two pH values. At pH 0·5, where 75% of the dye was in the

[1] Clark, W. M., and Perkins, M. E., *J. Am. Chem. Soc.*, 1932, **54**, 1228.
[2] Windholz, M., (Ed.) 'Merck Index' 9th Edn, p. 842 (Merck: Rahway, New Jersey, 1976).
[3] Hope, A. B., and Morland, A., *Aust. J. Plant Physiol.*, 1979, **6**, 289.
[4] Havemann, R., Pietsch, H., and Ollmann, D., *Z. Phys. Chem.* (*Leipzig*), 1960, **215**, 388.

0004-9425/83/030507$02.00

monoprotonated form and the rest diprotonated, the dye was photoreduced to form a yellow compound. The back-reaction with the Sn^{4+} ions produced followed only very slowly. At pH $-0\cdot5$, the reduction of Neutral Red was found to proceed in the dark with illumination increasing the rate of reaction.

Before the other explanations of the photosynthesis experiments which were proposed above can be properly examined, one must have a clear understanding of both the electrochemistry and the photoelectrochemistry of Neutral Red as a function of pH.

Bartels[5] has examined the pH dependence of the visible absorption spectrum of Neutral Red. He proposed four possible protonations of Neutral Red and gave the sites of protonation (in increasing difficulty of protonation) as: the ring nitrogen N 5, the dimethylamino group, the other ring nitrogen N 10, and finally the amino group. However, in solutions of pH > 0, only the singly protonated and unprotonated forms are present.[5]

Clark and Perkins[1] found several pH-dependent effects when Neutral Red was reduced by chromium(III) acetate in buffered solutions. The solution became almost colourless, but the original colour returned on exposure to air. When the reduced solution was left under N_2 or H_2, it

(i) remained colourless for longer than 1 h (pH $8\cdot2$), or

(ii) developed a yellow-green fluorescence very rapidly (pH $5\cdot3$), or

(iii) slowly acquired a yellow colour with a green fluorescence (pH $2\cdot7$).

More recently, Suzuki and Sawada[6] examined the reduction processes using d.c. and a.c. polarography, and cyclic voltammetry. The first wave in the polarogram had an $E_{1/2}$ of $-0\cdot47$ V (s.c.e.) at pH 5; the reaction was reversible and the product was fluorescent. A small wave which was attributed to the reduction of the product of the first reduction was seen at a more cathodic potential. This reaction was reversible also.

Nikol'skii *et al.*[7] have reported the pH dependence of the 'oxidation potentials' of the system Neutral Red–leuco-Neutral Red in the region $0\cdot5 \leqslant \text{pH} \leqslant 11\cdot5$. The data were divided into four linear segments with slopes as follows:

(A)	$0\cdot5 \leqslant \text{pH} \leqslant 4\cdot3$	-110 mV pH^{-1}
(B)	$4\cdot3 \leqslant \text{pH} \leqslant 6\cdot1$	-74 mV pH^{-1}
(C)	$6\cdot1 \leqslant \text{pH} \leqslant 7\cdot3$	-37 mV pH^{-1}
(D)	$7\cdot3 \leqslant \text{pH} \leqslant 11\cdot5$	-74 mV pH^{-1}

Segments (A)–(C) were displaced cathodically as the total concentration of dye was increased. The authors attributed this displacement to dimerization and tetramerization of the Neutral Red cation (the singly protonated form of the dye).

From calculations in an earlier paper,[8] Nikol'skii *et al.* considered the following equilibria

[5] Bartels, P., *Z. Phys. Chem.* (*Frankfurt am Main*), 1956, **9**, 74.

[6] Suzuki, M., and Sawada, S., *Denki Kagaku*, 1971, **39**, 249.

[7] Nikol'skii, B. P., Pal'chevskii, V. V., Polyanskaya, L. A., and Rodichev, A. G., *Dokl. Akad. Nauk SSSR*, 1970, **194**, 1334.

[8] Nikol'skii, B. P., Pal'chevskii, V. V., Polyanskaya, L. A., and Boriskin, V. V., *Dokl. Akad. Nauk SSSR*, 1970, **193**, 352.

$$RH^+ + H^+ + 2e^- \rightleftarrows RH_2 \quad (1)$$

$$RH^+ \rightleftarrows R + H^+ \quad (2)$$

$$RH_3^+ \rightleftarrows RH_2 + H^+ \quad (3)$$

$$RH_4^{2+} \rightleftarrows RH_3^+ + H^+ \quad (4)$$

where R represents the Neutral Red molecule and RH_2 represents the leuco-Neutral Red molecule. They determined that the pK of the deprotonation reactions were: 6·7 for reaction (2), 6·3 for reaction (3), and 4·4 for reaction (4).

By combining this information on the protonation of Neutral Red and leuco-Neutral Red, one has the following overall reactions for the reduction of Neutral Red in various pH regions

$$0{\cdot}5 < \text{pH} < 4{\cdot}4 \qquad RH^+ + 3H^+ + 2e^- \rightleftarrows RH_4^{2+} \quad (5)$$

$$4{\cdot}4 < \text{pH} < 6{\cdot}3 \qquad RH^+ + 2H^+ + 2e^- \rightleftarrows RH_3^+ \quad (6)$$

$$6{\cdot}3 < \text{pH} < 6{\cdot}7 \qquad RH^+ + H^+ + 2e^- \rightleftarrows RH_2 \quad (7)$$

$$6{\cdot}7 < \text{pH} < 11{\cdot}5 \qquad R + 2H^+ + 2e^- \rightleftarrows RH_2 \quad (8)$$

Reactions (5)–(7) involve the protonated cation but reaction (8) involves the Neutral Red molecule (note that pH 0·5 and 11·5 are only significant as the limits of experimental information).

Experimental

Neutral Red was obtained from Gurr (biological stain) and used as received. Five buffers were used: 0·1 M H_2SO_4 (pH 1·0), acetate buffer (pH 3·4), phthalate buffer (pH 5·0), phosphate buffer (pH 7·2) and borax buffer (pH 9·2). A.R. grade materials and Aristar sulfuric acid were used. The pH was adjusted with a sodium hydroxide solution (Volucon).

The specific conductances of the acetate and borax buffers were too low for electrochemical measurements, so a supporting electrolyte of 0·1 M K_2SO_4 was added.

Conventional three-compartment electrochemical cells were used in this work. Water was tri-distilled from an alkaline permanganate solution. A commercial saturated calomel electrode (Philips) was the reference electrode.

In photoelectrochemical measurements, light from a 900-W xenon lamp (Hanovia Lamp Division, Conrad Precision Ind.) was focused on the electrode after passing through a series of lenses, a water filter (7·3 cm long), and a long-pass filter (Oriel colour glass filter G-772-4750) which transmitted light of wavelength longer than 475 nm. The depth of solution between the glass window of the cell and the electrode was approximately 5 mm.

As with electrochemical measurements, an applied potential from a voltage scan generator (Wenking VSG72) was fed to a potentiostat (P.A.R. 173), but the current output was fed in the differential mode into a lock-in amplifier (P.A.R. HR-8). The reference signal was provided by a mechanical light chopper (P.A.R. 126A). The output of the voltage scan generator and the lock-in amplifier were monitored with an X–Y recorder (Houston Omnigraph 2000, type 5 input module).

Other experimental details have been presented elsewhere.[9]

Electrochemistry

Linear potential scans were used to examine the electrochemistry of the dye on vitreous carbon and copper electrodes. When solutions of Neutral Red in each buffer were scanned with a vitreous carbon electrode, it was found that Neutral Red underwent pH-dependent oxidation and reduction reactions. By scanning anodically and

[9] Halliday, C. S., Ph.D. Thesis, The Flinders University of South Australia, 1981.

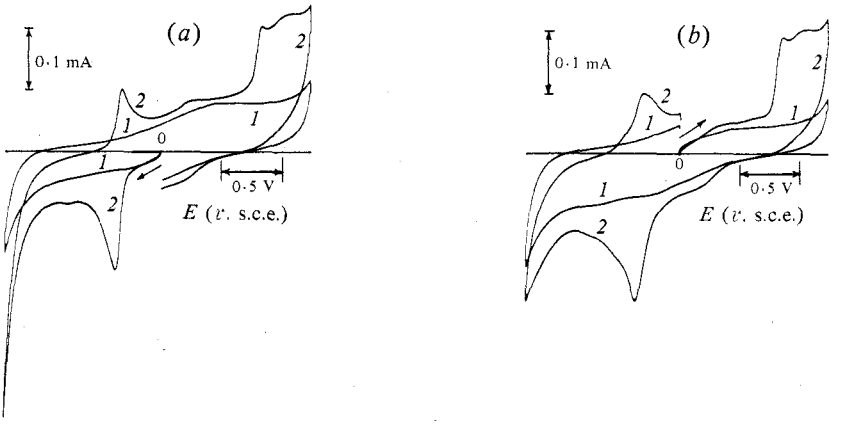


Fig. 1. Linear potential scan voltammograms [from −1·262 to +1·237 V (s.c.e.), pH 3·4]: vitreous carbon/acetate buffer, Neutral Red; scan rate 100 mV s^{-1}. (*a*) Cathodic; (*b*) anodic from 0 V. *1*, Without Neutral Red; *2*, with 1 mM Neutral Red.

Table 1. Peak potentials at a scan rate of 100 mV s^{-1}

a, anodic peak; c, cathodic peak. Errors in E_p and $E_{p/2}$ values: with three significant figures, ±10 mV; with two significant figures, ±20 mV

pH	Buffer solution	Electrode	E_p/V (s.c.e.)	$E_{p/2}$/V (s.c.e.)	ΔE_p/mV	Dye concn/mM
1·0	0·1 M H_2SO_4	vitreous carbon	−0·170(c),[A] −0·120(a),[A] +1·015(a), +1·165(a)	+0·930(a) −0·100(c)	50	0·2
		copper disc	−0·120(c),[A] −0·165(c), −0·090(a)[A]	−0·095(c)	30	0·3
		platinum disc	−0·145(c),[A] −0·105(a),[A] +0·950(a)	+0·90(a) −0·110(c)	40	0·3
3·4	acetate	vitreous carbon	−0·750(c), −0·370(c),[A] −0·305(a),[A] +0·20(c), +0·850(a), +1·15(a)	+0·80(a) −0·305(c)	65	1
		copper disc	−0·71(c), −0·340(c),[A] −0·300(a)[A]	−0·310(c)	40	1
5·0	phthalate	vitreous carbon	−0·860(c), −0·510(c),[A] −0·430(a),[A] +0·10(c), +0·20(a), +1·1(a)	+0·88(a) −0·410(c)	80	0·1
		copper disc	−0·90(c), −0·525(c),[A] −0·420(a)[A]	−0·430(c)	105	0·1
7·2	phosphate	vitreous carbon	−0·960(c), −0·635(c),[A] −0·550(a),[A] +0·85(a)	+0·70(a) −0·570(c)	85	0·2
		platinum disc	−0·635(c),[A] −0·550(a),[A] +0·80(a)	−0·59(c)	85	0·4
9·2	borax	vitreous carbon	−0·77(c),[A] −0·68(a),[A] +0·71(a)	+0·60(a) −0·70(c)	90	[B]
		copper disc	−1·07(c), −0·970(c), −0·830(c),[A] −0·735(a)[A]	−0·750(c)	95	[B]

[A] ΔE_p obtained from these peaks. [B] Saturated solution.

cathodically from 0 V (s.c.e.), it was seen that the first oxidation and reduction reactions were those of the parent molecule (e.g., see Fig. 1).

Table 1 lists the peak potentials at a scan rate of 100 mV s^{-1} for all peaks seen on various electrode materials in the five buffer solutions used. The data were averaged from at least two scans. For the first anodic and cathodic peaks seen in scanning from 0 V (s.c.e.), $E_{p/2}$ values are listed as are ΔE_p values for linear potential scan voltammograms for the reduction of Neutral Red.

The scan rate dependence of the peak current (i_p) for the first reduction of Neutral Red in solutions of various pH was examined with a vitreous carbon and a copper electrode. Plots of $\log i_p$ against $\log v$ (where v is the scan rate) were linear with a slope of 0·5. This corresponds to the theoretical value for a simple electrochemical reaction.[10]

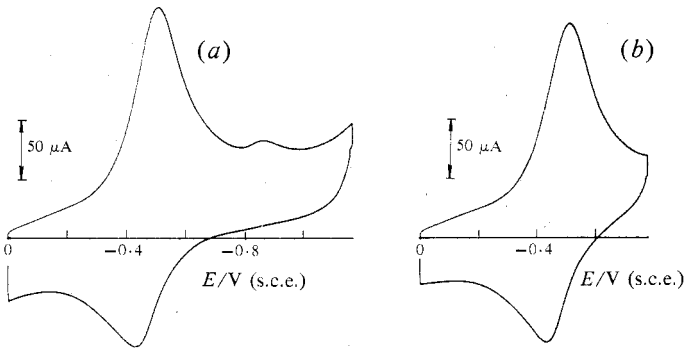


Fig. 2. Effect of cathodic limit on linear potential scan voltammograms of 10^{-4} M Neutral Red (pH 5·0): vitreous carbon/phthalate buffer; scan rate 100 mV s^{-1}. Cathodic from 0 V to: (*a*) −1·170 V (s.c.e.); (*b*) −0·781 V (s.c.e.).

In acetate buffer (pH 3·4) the linear potential scan voltammogram on vitreous carbon illustrates most of the features of the voltammograms observed at other pH. In Fig. 1*a* it can be seen that two reductions were observed when the potential was scanned cathodically from 0 V (s.c.e.). For the first reduction and the oxidation of its product, ΔE_p was close to the theoretical value for a one-electron, reversible reaction. This was clearly seen in the response on vitreous carbon in phthalate buffer (Fig. 2).

These two reduction peaks were clearly seen in the linear potential scan voltammogram (Fig. 3) on a copper electrode in borax buffer. Also, another reduction peak was revealed with the cathodic shift of hydrogen evolution with increasing pH. The reoxidation was independent of the cathodic limit.

When the potential was scanned anodically from 0 V (s.c.e.) on vitreous carbon, the linear potential scan voltammogram showed two oxidation peaks and a reduction peak on the reverse cycle (Fig. 1*b*). The latter was broad and approximately 0·65 V cathodic of the first oxidation. By applying Tomes criterion[11] to the first oxidation wave, it was found that $|E_{1/4}-E_{3/4}|$ was 60 mV. This value is that which corresponds to a one-electron, reversible reaction.

[10] Nicholson, R. S., and Shain, I., *Anal. Chem.*, 1964, **36**, 706.
[11] Fry, A. F., 'Synthetic Organic Electrochemistry' (Harper & Row: New York 1972).

Plots of $\log i$ against E on vitreous carbon at pH 1·0 and 7·2 are given in Fig. 4. The Tafel slopes are 60 mV per decade which is the theoretical value for a one-electron reversible reaction. The poor solubility of Neutral Red in aqueous solution limited the width of the region between the background current and the diffusion-limited current.

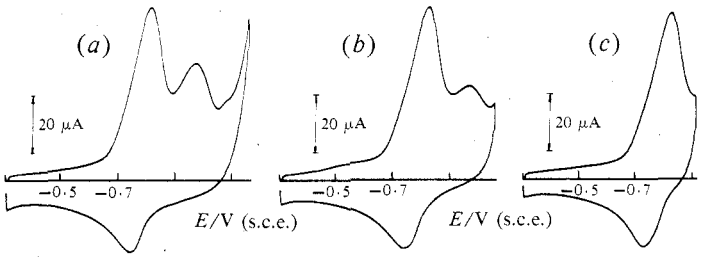


Fig. 3. Effect of cathodic limit on linear potential scan voltammograms of Neutral Red (saturated solution, pH 9·2): copper disc/borax buffer. Cathodic from −0·319 V (s.c.e.) to: (*a*) −1·169 V (s.c.e.); (*b*) −1·061 V (s.c.e.); (*c*) −0·919 V (s.c.e.).

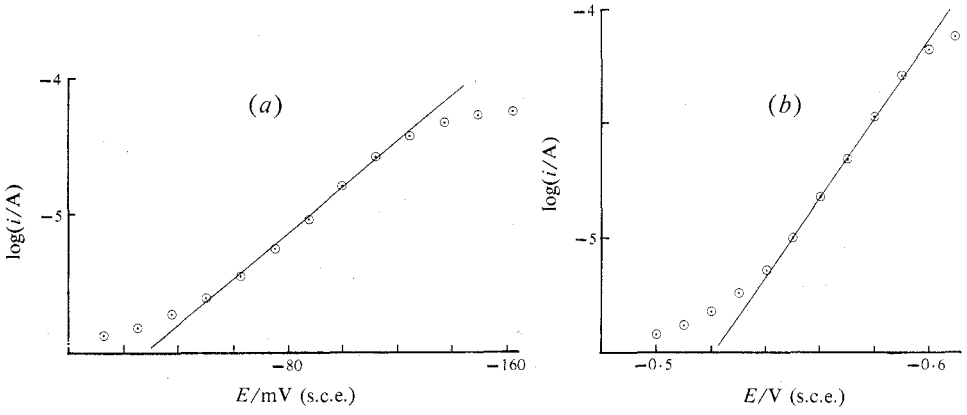


Fig. 4. Plots of $\log i$ against potential on vitreous carbon for 10^{-4} M Neutral Red in: (*a*) 0·1 M H_2SO_4 (pH 1·0, scan rate 1 mV s^{-1}); (*b*) phosphate buffer (pH 7·2, scan rate 5 mV s^{-1}).

Discussion

The $E_{p/2}$ values in Table 1 for the oxidation and the reduction reactions are plotted in Fig. 5 as a function of pH. Where the second reduction peak was observed, E_p values are plotted on the same graph.

The *oxidation* reaction exhibited a linear dependence on pH over the range examined with a slope of −60 mV pH^{-1}. Hence H^+ ions were released in the reaction with an e^-/H^+ ratio of one. Neutral Red underwent a one-electron, reversible reaction with the loss of one proton. Thus, if the Neutral Red molecule is represented by AH and the protonated form by AH_2^+, above pH 6·7

$$AH \rightleftarrows A + H^+ + e^- \tag{9}$$

and below pH 6·7

$$AH_2^+ \rightarrow AH^+ + H^+ + e^- \tag{10}$$

The second oxidation reaction observed at pH 1·0 and 3·4 had approximately the same E_p value for each linear potential scan voltammogram. At pH 5·0 this second oxidation was hidden in the broad oxidation wave. At pH 7·2 this second peak was not seen. This may be because the pH of the solution was above the pK_a of Neutral Red and hence the protonated Neutral Red molecule was not present in significant concentration. That is, the second wave is an oxidation of the species AH_2^+

$$AH_2^+ \rightarrow AH_2^{2+} + e^- \tag{11}$$

or its first oxidation product AH^+

$$AH^+ \rightarrow AH^{2+} + e^- \tag{12}$$

Neither reaction involved the gain or loss of protons.

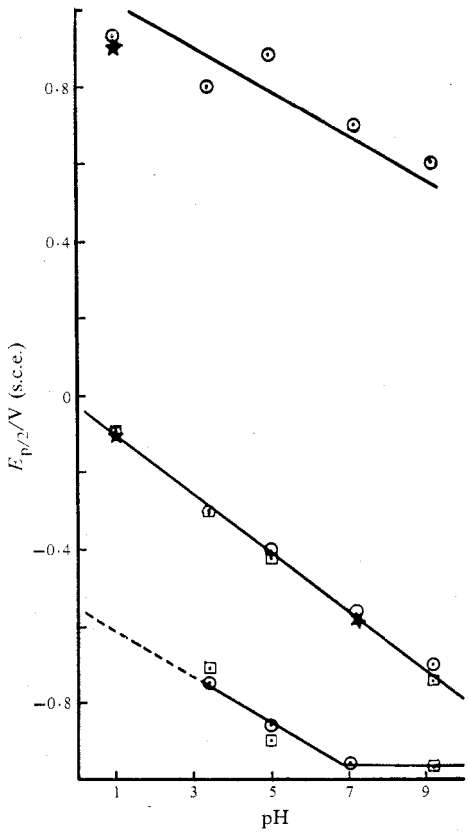


Fig. 5. Half-peak potentials ($E_{p/2}$) against pH for Neutral Red.
o Vitreous carbon electrode;
□ copper electrode;
★ platinum electrode.

Returning to Fig. 5 and examining the pH dependence of the first *reduction* (-77 mV pH^{-1}), one finds an e^-/H^+ ratio of one. With the notation of equations (1)–(4), and since one electron is reversibly consumed per Neutral Red molecule, the reduction reaction can be written as

$$R + H^+ + e^- \rightleftarrows RH^{\bullet} \tag{13}$$

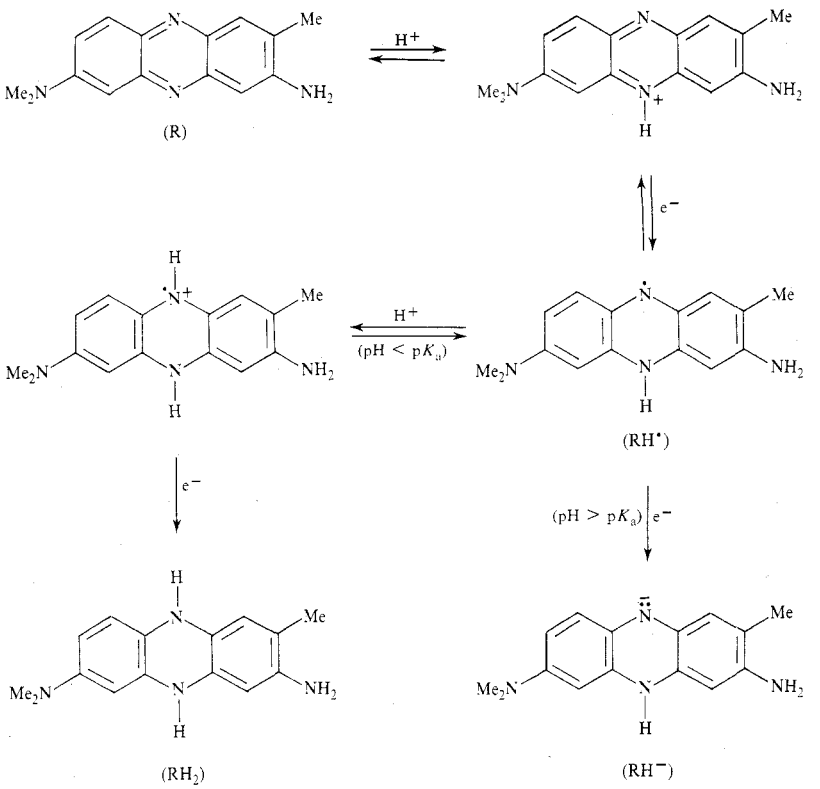
Below the pK_a of either R or RH_2 [see equations (2) and (3)], the pH dependence of E_p for the second reduction suggests an e^-/H^+ ratio of one. Thus the reaction of the first reduction product for $pH < pK_a$ is

$$RH^{\bullet} + H^+ + e^- \rightarrow RH_2 \tag{14}$$

Above pK_a the reaction is

$$RH^{\bullet} + e^- \rightarrow RH^- \tag{15}$$

Scheme 1 shows the molecular reactions based on Bartels's assignment[5] of the ease of protonation of the various sites of the Neutral Red molecule and the product suggested by Nikol'skii *et al.*[7] The product RH^- may be protonated after the electron-transfer step at $pH > pK_a$.



Scheme 1

A comparison of equations (5)–(8) and the reactions up to and including the electron-transfer step in Scheme 1 suggests the following points: at $pH > pK_a$ an H e e H reaction mechanism is operative; and at $pH < pK_a$ an H e H e mechanism is followed by another one ($6{\cdot}3 < pH < 4{\cdot}4$) or two ($4{\cdot}4 < pH < 0{\cdot}5$) protonation reactions.

In the results of Clark and Perkins[1] which were summarized in the introduction to this paper, it is interesting to note that the three pH values which they used to examine the appearance of a green fluorescence fall in the middle of the pH regions

proposed[7] for leuco-Neutral Red [equations (3) and (4)]. It may be that the species RH_3^+, which is formed from the reduction product, leuco-Neutral Red, by the addition of a proton to the dimethylamino group, fluoresces (pH 5·3).

Photoelectrochemistry

In Fig. 6*b* the photocurrent scan with Neutral Red in phosphate buffer (pH 7·2) on a platinum electrode is shown. This scan remained essentially unchanged for the first five cycles measured. The scan in Fig. 6*a* is the cyclic voltammogram in the dark between the same potential limits. The anodic peak at about +0·73 V (s.c.e.) in this voltammogram is that due to oxidation of Neutral Red. The reduction of Neutral Red was obscured by hydrogen evolution.

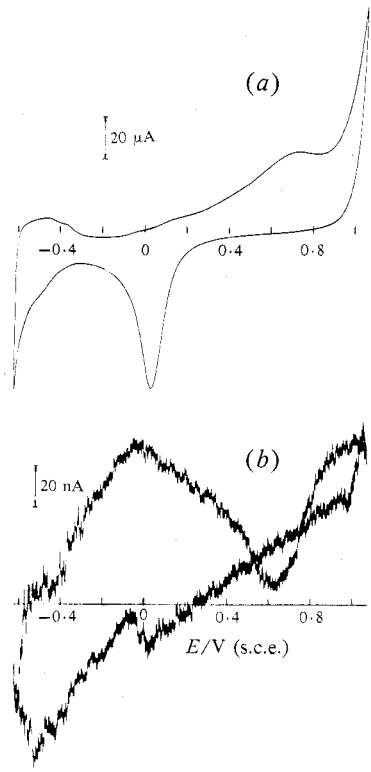


Fig. 6. Platinum disc/1 mM Neutral Red, phosphate buffer (pH 7·2). (*a*) Steady-state cyclic voltammogram in dark; (*b*) first cycle of photocurrent scan. Scan rate 10 mV s^{-1}.

In the photocurrent scan of Fig. 6*b*, a broad, anodic peak centred at about 0 V (s.c.e.) was seen. The photocurrent diminished as the dark reaction commenced. This would result from the decreasing surface concentration of Neutral Red. At potentials more positive than about +0·6 V (s.c.e.) the photocurrent increased. This may be attributed to an interaction of the excited dye with the 'platinum oxide' substrate. The broad, cathodic peak centred at about −0·5 V (s.c.e.) is complicated by the presence of hydrogen evolution. This effect of hydrogen evolution on the photocurrent is supported by the fact that no cathodic photocurrent was observed with a copper electrode in the same potential region. In the linear potential scan voltammogram on copper the anodic limit was approximately −0·1 V (s.c.e.) and hydrogen evolution was seen cathodic of approximately −1 V (s.c.e.).

When methylviologen was added to the system, the photocurrent scan was the same as that shown in Fig. 6*b*.

When Neutral Red was examined with n-type semiconductor electrodes, no photocurrent was observed. The flat-band potentials of the conduction bands of the three semiconductors examined, n-$SrTiO_3$, n-TiO_2 and n-GaP, are all cathodic of 0 V (s.c.e.). Thus the results agree with the response obtained with the platinum electrode: the excited dye could not be made to act as an excited electron donor to acceptor levels more cathodic than approximately 0 V (s.c.e.).

Conclusions

The pH of biological media falls into the region for which the reduction of Neutral Red is described by equation (8), and the oxidation of the dye is described by equation (9). Moreover, it has been shown that at this pH the overall reaction (8) proceeds by an H e e H mechanism. From the data shown in Fig. 6 and a knowledge of the E° values of the reactions which are present in a photosynthetic system being studied, it is possible to predict whether Neutral Red will act as an artificial electron donor or acceptor. Thus, the dye will appear to be lost from the system if its presence is measured by absorption.

The photoelectrochemical examination of Neutral Red has shown that, provided an appropriate electron-acceptor level is available, the excited dye can be oxidized. Again if the E° values of the various reactions occurring in a photosynthetic system are appropriate, Neutral Red may be lost from the system through a photoelectrochemical reaction.

Acknowledgments

We wish to thank Dr S. Fletcher for his helpful discussions and Professor A. B. Hope for drawing our attention to the problem. One of us (C.S.H.) acknowledges the support of a Commonwealth Postgraduate Research Award.

Manuscript received 6 May 1982