

ELECTROCHEMICAL BEHAVIOUR OF NEUTRAL RED

A NIXON AZARIAH, SHEELA BERCHMANS AND V YEGNARAMAN*

Central Electrochemical Research Institute, Karaikudi - 630 006, INDIA

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The electrochemical studies of the dye molecule, Neutral Red (NR) assume importance in view of its redox characteristics. The oxidised and reduced forms of NR, depending upon the solution pH, undergo different protonation reactions. The electrochemical behaviour of NR (3-amino-7-dimethylamino-2-methyl phenazine) has been studied voltammetrically on a glassy carbon electrode at different pH conditions. The investigations indicate that the electroreduction of NR follows a single stage $2e^-$ transfer. Based on the voltammetric data, the diffusion coefficient of NR and the heterogeneous rate constant for its reduction reaction have been estimated. The results of the above investigations are presented and discussed in this communication which also includes absorption spectral data of NR in the visible range.

Keywords: Voltammetry, neutral red, electroreduction, absorption spectra.

INTRODUCTION

The dye molecule, 3-amino-7-dimethylamino-2-methyl phenazine, popularly called Neutral Red (NR), has long been used as a pH indicator in biological studies because of its red- yellow colour change at a pKa value of 6.7 which falls in the pH range close to that of biological media [1]. The structure (Fig. 1) and the electrochemical property of NR are closely related to flavins [2]. Hence NR can be considered as a model compound for the investigations on flavoenzymes and also as a redox indicator for electrochemical investigations of biological systems.

NR is a derivative of phenazine whose electrochemistry has been well studied. Phenazines are highly reversible systems which can act as e^- transfer mediators [3,4]. The electrochemistry of NR, in view of its biological and industrial importance, has been an active area of research and investigations on the electrochemical reduction of NR have been reported [5-7]. In aqueous solution, NR is reported [5] to undergo reduction in two stages each involving a single electron transfer. It has also been reported [6] that NR undergoes a single stage reduction, which involves $2e^-$ transfer. Further, the reduction reaction is described [7] to

be complicated by the existence of different ionic forms of NR at different pH values. To throw further light, voltammetric investigations of the reduction of NR at different pH values, supported by spectrophotometric methods were undertaken and the results are described in this communication.

EXPERIMENTAL

Voltammetric experiments were carried out in a conventional three-electrode glass cell with a glassy carbon disc (3 mm dia.) as the working electrode. A platinum foil was the counter electrode and a normal calomel electrode (NCE) served as the reference against which all potential values in this study are referred to. Prior to the experiments, the working electrode was polished using 1/0, 2/0, 3/0, 4/0 emery papers (John Oakey & Sons, London) and alumina slurry

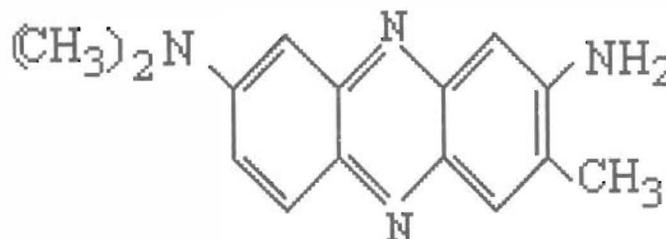


Fig. 1: Structural formula of Neutral Red

* Author for correspondence

Calculation of heterogeneous rate constant

As discussed earlier, the CVs obtained for the reduction of NR follow a similar pattern at different pH values studied. An analysis of the CVs obtained at various pH and different scan rate conditions yields the following characteristic features.

1. With increase in scan rate, E_{pc} shifts cathodically and E_{pa} anodically.
2. ΔE_p is around 25 to 30 mV at low scan rate and it gradually increases with the increase in scan rate.
3. Plots of i_{pc} or i_{pa} vs. $v^{1/2}$ is linear in all the pH ranges studied.
4. At all scan rates, $(i_{pc}/i_{pa}) > 1$.

From the above results it can be inferred that the electron transfer under the present experimental conditions is quasi reversible. It is quite common for a process that is reversible at low sweep rates to become irreversible at higher ones after having passed through a region known as quasi reversible at intermediate values. This transition from reversibility to quasi-reversibility and then to irreversibility occurs when the relative rate of the electron transfer with respect to that of mass transport is insufficient to maintain Nernstian equilibrium at the electrode surface. In the quasireversible region, both forward and backward reactions make a

TABLE I: Voltammetric data of Neutral Red at different pH and scan rates

pH	SR V/s	E_{pc} V	E_{pa} V	ΔE_p mV	i_{pc} μA	i_{pa} μA	i_{pc}/i_{pa}
2	0.003	-0.255	-0.230	25	3.40	2.52	1.34
	0.005	-0.255	-0.225	30	4.40	3.26	1.35
	0.010	-0.260	-0.225	35	6.80	5.00	1.36
	0.020	-0.260	-0.225	35	8.80	6.40	1.37
	0.030	-0.265	-0.225	40	10.20	7.30	1.39
	0.040	-0.265	-0.225	40	12.20	8.60	1.42
	0.050	-0.265	-0.220	45	13.80	9.50	1.45
	0.080	-0.270	-0.220	50	17.30	11.88	1.46
	0.160	-0.275	-0.215	60	24.42	15.84	1.50
	0.200	-0.280	-0.210	70	26.40	16.83	1.57
4	0.300	-0.290	-0.200	90	31.02	19.15	1.62
	0.500	-0.295	-0.190	105	40.92	24.09	1.70
	0.003	-0.420	-0.395	25	3.30	2.35	1.40
	0.005	-0.425	-0.395	30	4.50	3.20	1.40
	0.010	-0.425	-0.390	35	6.60	4.30	1.41
	0.020	-0.425	-0.385	40	8.20	5.70	1.43
	0.030	-0.430	-0.382	45	9.70	6.65	1.46
	0.040	-0.430	-0.380	50	11.00	7.60	1.45
	0.050	-0.440	-0.380	60	13.10	9.00	1.46
	0.080	-0.440	-0.375	65	15.20	10.40	1.46
5	0.160	-0.445	-0.360	85	21.60	14.00	1.54
	0.200	-0.450	-0.360	90	24.40	15.40	1.58
	0.300	-0.455	-0.355	100	27.50	17.16	1.60
	0.500	-0.460	-0.350	110	38.94	22.44	1.74
	0.003	-0.520	-0.495	25	4.40	2.30	1.90
	0.005	-0.525	-0.495	30	6.00	3.30	1.82
	0.010	-0.525	-0.492	33	7.60	3.30	1.81
	0.020	-0.525	-0.490	35	9.00	5.00	1.80
	0.030	-0.525	-0.485	40	14.60	7.80	1.83
	0.040	-0.525	-0.480	45	15.00	8.40	1.80
8	0.050	-0.530	-0.480	50	17.60	10.00	1.76
	0.080	-0.530	-0.475	55	22.00	13.00	1.70
	0.160	-0.535	-0.470	65	36.30	21.78	1.67
	0.200	-0.540	-0.465	75	37.62	23.10	1.63
	0.300	-0.545	-0.460	85	44.90	29.70	1.51
	0.500	-0.560	-0.455	105	55.44	37.62	1.47

Table I continued

TABLE I: Voltammetric data of Neutral Red at different pH and scan rates

pH	SR V/s	E_{pc} V	E_{pa} V	ΔE_p mV	i_{pc} μA	i_{pa} μA	i_{pc}/i_{pa}
6	0.003	-0.565	-0.540	25	3.00	1.65	1.82
	0.005	-0.565	-0.540	25	4.60	2.40	1.92
	0.010	-0.570	-0.540	30	7.00	3.65	1.92
	0.020	-0.570	-0.535	35	8.40	4.50	1.90
	0.030	-0.575	-0.535	40	11.40	6.60	1.73
	0.040	-0.580	-0.535	45	12.60	7.40	1.70
	0.050	-0.580	-0.530	50	14.50	8.60	1.69
	0.080	-0.585	-0.530	55	17.60	10.60	1.67
	0.160	-0.590	-0.525	65	28.05	17.50	1.60
	0.200	-0.595	-0.520	75	30.36	19.80	1.53
8	0.300	-0.600	-0.515	85	40.26	26.60	1.51
	0.500	-0.610	-0.510	100	52.80	34.98	1.50
	0.003	-0.645	-0.620	25	4.50	2.60	1.73
	0.005	-0.645	-0.615	30	5.60	3.30	1.69
	0.010	-0.650	-0.615	33	9.00	5.40	1.67
	0.020	-0.650	-0.613	37	13.60	8.60	1.58
	0.030	-0.650	-0.610	40	17.20	11.20	1.54
	0.040	-0.655	-0.610	45	19.60	13.40	1.46
	0.050	-0.655	-0.605	50	22.40	15.20	1.47
	0.080	-0.655	-0.600	55	29.70	21.12	1.41
8	0.160	-0.660	-0.595	65	47.52	31.68	1.50
	0.200	-0.660	-0.590	70	53.00	34.98	1.50
	0.300	-0.670	-0.580	90	69.00	47.50	1.45
	0.500	-0.680	-0.570	110	93.00	65.00	1.43

contribution to the observed current and the region is generally recognized to have the following boundaries [9].

$$0.3 > k_h^0 > 2 \times 10^{-5} \text{ cm/s.}$$

Where k_h^0 is the heterogeneous rate constant. The kinetics of quasi-reversible electron transfer can be studied using Nicholson's method [10].

The diffusion coefficient of NR (D_{NR}) was estimated [11] using the following equation

$$(D_{NR})^{1/2} = \frac{i_{pc}}{2.69 \times 10^5 n^{3/2} A v^{1/2} C_{NR}} \quad (1)$$

where 'n' is the number of electrons transferred, A is the true surface area of the working electrode and C_{NR} is the concentration of NR in solution.

The true surface area of the working electrode was estimated from the reversible CV obtained on the electrode for the ferro/ferricyanide redox system and using the Randles-Sevcik equation [12]. For this purpose, a CV was recorded on the same electrode at 0.020 V/s in 0.1 M KCl solution containing 5 mM of $K_4Fe(CN)_6$. From the CV data, the true surface area is found to be $7.82 \times 10^{-2} \text{ cm}^2$ (as against the geometric area of $7.071 \times 10^{-2} \text{ cm}^2$). Using the above value of 'A' and the CV data obtained for NR at low scan rates where the reversibility of the system is well-marked, the D_{NR} was estimated to be $1.90 \pm 0.2 \times 10^{-6} \text{ cm}^2/\text{s}$.

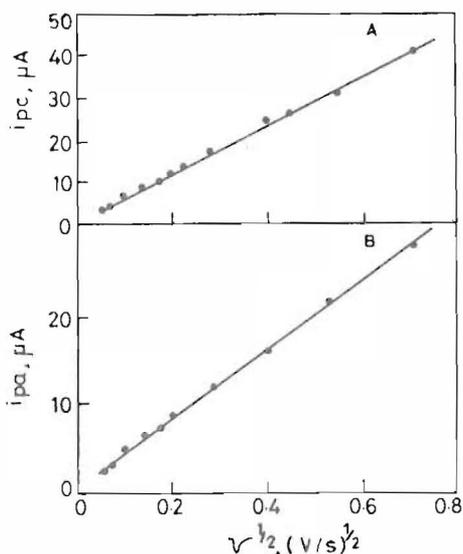


Fig. 3: i_p vs $v^{1/2}$ plot of Neutral Red at pH = 2
(A) = Cathodic and (B) = Anodic

It is known [10] that ΔE_p is a function of ψ and is further related to k_h^0 by the following expression

$$\psi = \frac{r^\alpha k_h^0}{(\pi n D_{NR} F v)^{1/2}} \quad (2)$$

where 'α' and 'r' denote transfer coefficient and (D_{ox}/D_R) respectively and the value of 'r' is assumed to be one for k_h^0 calculations.

From the estimated value of D_{NR} and the ψ values from tables [10,13], the k_h^0 values are fairly high at all pH, approaching near reversibility of the redox behaviour. The tendency for the k_h^0 to be a little lower at pH=8, could be attributed to the reduction of neutral species at this pH, while at all other pH values studied, protonated species are involved in the reduction reaction.

Reduction behaviour of NR

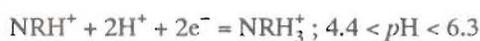
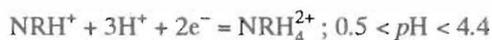
As remarked earlier, E_{pc} values shift cathodically as pH is increased (Cf. Table I). This obviously shows that the redox behaviour of the system under study is significantly influenced by the solution pH, through protonation reactions. The phenazine/dihydrophenazine system, responsible for the redox characteristics of the NR, exhibits, in water and other protic media, a series of protonation equilibria and consequently pH-dependent redox behaviour [3,4,14]. The oxidized form of NR is reported to undergo four possible protonation reactions depending upon the solution pH [15].

The present results suggest that NR undergoes reduction to NRH_2 through a single stage $2e^-$ transfer reaction, which agrees with the earlier reports [14]. This follows naturally from the fact that the azobenzene molecule which is the redox-active centre in phenazine and NR molecules, undergoes a single stage $2e^-$ transfer reaction [16].

The following over-all reactions for the reduction of NR at different pH have been reported [5]

TABLE II: Heterogeneous rate constant (k_h^0) of Neutral Red at different pH values

pH	2	4	5	6	8
k_h^0	0.168 ± 0.01	0.182 ± 0.01	0.182 ± 0.01	0.182 ± 0.01	0.132 ± 0.01



At acidic pH, the oxidised form exists only as mono protonated ones. It takes up two electrons and attains the reduced form of the Neutral Red molecules. The reduced molecule gets further protonated depending upon the pH of the medium. The reduction is believed to take place as a single stage 2e^- transfer reaction in the aqueous medium. The reduction of NR is also suggested to proceed through two successive single electron transfer steps as predicted by a square scheme [17]. However, in protic media, this possibility is very remote since the formation of radical anions will be highly impeded due to their very poor stability.

The above discussions point to the reduction of NR through a single stage two electron transfer reaction. The reduced NR molecule will readily undergo a succeeding chemical reaction, viz., protonation. The rate of the oxidation of the protonated molecules during the reverse potential scan will be expected to be slower when compared to that of the

TABLE III: Spectral data of Neutral Red in different pH solutions

pH	λ -max	Intensity of abs
2	538.00	1.8220
4	521.28	1.6356
5	519.76	1.5320
6	517.40	1.3469
8	454.56	0.9110

unprotonated species. This will result in a lower i_{pa} value for the protonated molecule compared to that for the unprotonated species. The results of the present investigations show that at all pH values, i_{pa} is found to be less than the i_{pc} values, thereby indicating the influence of the succeeding protonation reaction on the CV behaviour.

Spectral behaviour of NR at different pH

The visible absorption spectra of NR in McIlvane's buffer solutions is interesting (Fig. 4). Absorption maxima (λ -max) and the intensity of absorption gradually decreased with increase in the pH of the background electrolyte which is analogous to the behaviour reported for methylene blue [18]. The spectral data is given in the Table III. At pH=8, there is a sharp decrease in the values of λ -max and the intensity of absorption.

Like methylene blue, NR also has N-atoms in the chromophore (phenazine ring) which may be easily protonated [6]. The N-atom has a very little tendency to act as an acid when compared to carbon [19]. When proton is added to the central nitrogen atom any characteristic charge remaining on the nitrogen is driven into the rest of the molecule. Thus bathochromic shift [red shift] results. Towards alkaline pH, blue shift results. When compared with other pH, λ -max and intensity of absorption are lowered to a greater extent at pH=8. This could be attributed to the alkaline conditions in which NR is predominantly present in the leuco form and hence λ -max and the intensity of absorption are appreciably decreased.

CONCLUSION

The electrochemical behaviour of Neutral Red (3-amino-7-dimethylamino-2-methyl phenazine) was studied voltammetrically in solutions of different pH (pH=2,4,5,6 & 8). At all pH conditions, the compound exhibits near-reversible redox behaviour and the heterogeneous rate constant values are found to be around 0.15 cm/s. The

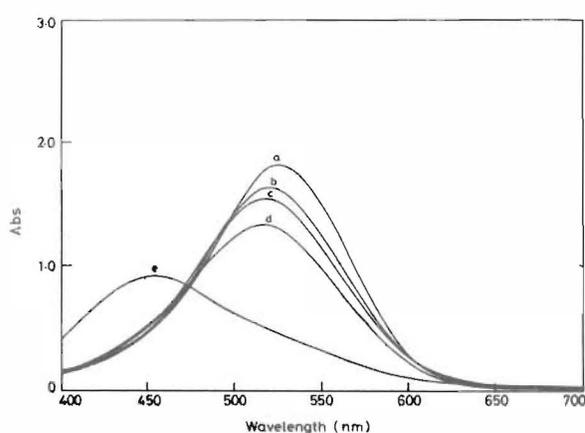


Fig. 4: Visible absorption spectra of Neutral Red (0.01 mM) in McIlvane's buffer of pH (a) 2 (b) 4 (c) 5 (d) 6 (e) 8

reduction of the molecule follows a single stage $2e^-$ transfer which agrees with earlier reports. From the voltammetric results, the diffusion co-efficient is estimated to be $1.90 \pm 0.2 \times 10^{-6} \text{ cm}^2/\text{s}$. Visible absorption spectral studies have shown that, with the increase of pH, the absorption maximum and the intensity of absorption gradually decrease in the acidic range, with a relatively rapid decrease at pH=8.

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