ADSORPTION AND ACCUMULATION OF NEUTRAL RED AT MERCURY/SOLUTION INTERFACE

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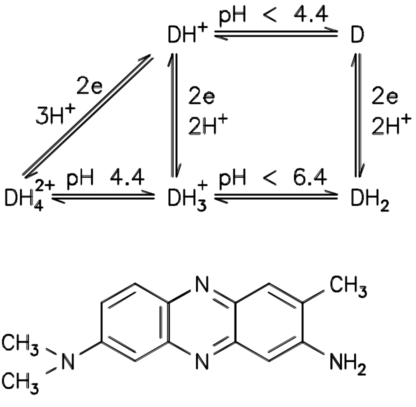
The voltammetric behaviour of protonated and nonprotonated neutral red (NR) has been studied at a hanging mercury drop electrode (HMDE) by using alternating current (AC) and cyclic voltammetry (CV). The AC voltammetric behaviour and the morphology of the adsorption redox peaks indicate that interactions between adsorbed molecules are substantial and that the nature of these interactions changes from moderately repulsive below pH 4 to strongly attractive above pH 6. Controlled adsorptive accumulation of NR on the HMDE provides the basis for the direct stripping measurement of that compound in the nanomolar concentration level. Adsorptive phase selective AC stripping voltammetry has been proved to be advantageous over any other assay technique, allowing a detection limit of 1.0 . 10^{-9} M NR to be reached.

Neutral red (NR) is one of the great number of organic dyes which are known as biological stains and are often used in biology, pharmacology and medicine. Previously reported potentiometric, polarographic and voltammetric studies[1–10] show a variety of values of the p*K*′s of the oxidized (D) and reduced (DH_2) forms of NR. The overall reaction mechanism can be summarized in Scheme 1 (ref.[10]). It is clear that the H^+ concentration plays an important role on both oxidation and reduction processes of NR.



SCHEME 1

D

The electrochemical behaviour of neutral red has been examined at the mercury electrode surface using DC, AC polarography[8,9] and linear sweep voltammetry[10] (LSV). The LSV measurements[10] have shown that the acidic or basic forms of both oxidized and reduced species of neutral red are strongly adsorbed at the mercury electrode surface.

Therefore, the present work was done to study the behaviour of the adsorbed protonated species (pH 2.0 – 6.2) and neutral species (pH > 6.4) of NR at the mercury/solution interface in solutions of varying pH. The effective adsorption of the compound on the HMDE was demonstrated, and it constitutes the basis for an adsorption stripping assay of the molecule. The AC stripping voltammetry of the adsorbed NR appeared to be the most sensitive analytical method. Thus, linear calibration plots could be extended by preconcentration of NR under open circuit conditions.

EXPERIMENTAL

Apparatus

Cyclic voltammetric experiments were carried out using a Model 303 HMDE in conjunction with a PAR Model 173 potentiostat and PAR 175 universal programmer. Differential pulse (DP) and AC stripping voltammetry were done using a Model 303 coupled to a PAR Model 174A polarographic analyzer. Voltammograms were recorded on a Hewlett–Packard 7045 X-Y recorder. The cell was maintained at 22 ± 0.5 °C. An Ag/AgCl/saturated KCl reference electrode and a Pt wire auxiliary electrode were used in a three-electrode system. A stirr-head bar and a PAR Model 305 stirrer were also used for AC stripping measurements.

Reagents and Procedures

Neutral red obtained from B.D.H. Laboratory Chemicals Division (England) was used without further purification. Stock solutions ($1 . 10^{-4}$ mol l^{-1}) were prepared and kept in the dark. Solutions of 0.4 M Britton–Robinson buffer adjusted to a constant ionic strength with NaCl were used as background electrolytes in the pH region 2 – 12. Twice distilled deionized water served as the solvent. The pH was measured with a digital Radiometer pH-meter Model M64 with the accuracy of ± 0.05 pH units.

Test solutions were transferred to the cell and oxygen was removed by passing a stream of oxygen-free nitrogen for 15 min. Sequential additions of stock solutions of NR were used to obtain the calibration curves and test mixture data. Statistical analysis of calibration curve parameters is included.

Computation performed on an Apple IIe microcomputer was based on unweighted linear least square fits.

RESULTS AND DISCUSSION

Adsorption of Protonated and Nonprotonated NR Studied Using AC and Cyclic Voltammetry

The study of the voltammetric behaviour of adsorbed NR species at the mercury electrode surfaces in solution of varying pH was carried out at the conditions which allowed to enhance the NR adsorption current relative to the diffusion-controlled ones.

The CV response of a fresh mercury electrode in 5 μM NR solution at pH 3.2 is shown in Fig. 1. In the pH range of 2 – 6.2, both the oxidized (D) and reduced (DH_2) forms of the phenazine system are cations (Scheme 1). In this pH range the electrode potential did not exceed the value negative of −0.4 V, so that the mercury surface can be considered as positively charged. The coulombic interactions among adsorbed protonated species (in reduced or oxidized forms) as well as interactions with the electrode surface are most probably repulsive. The CV shows a small and broad peak couple (Fig. 1). The peak shape may indicate higher repulsive forces between the DH_4^{2+} species at the charged electrode, which has been confirmed also by means of the out-of-phase AC measuremets. The AC voltammograms are characterized by a pseudocapacitive peak split (Fig. 2) at higher frequencies, assigned to reduction of DH^+ to DH_4^{2+}. The peak split disappears at frequencies below 200 Hz or at lower concentrations. A progressive decrease of the capacitive AC signal at the electrocapillary zero charge of the pure supporting electrolyte corresponds to coverage of the electrode surface by the adsorption layer[11,12] of NR with the phenazine system oriented parallel to the electrode surface[13–15].

The adsorption behaviour of neutral oxidized and reduced forms at a fresh HMDE surface has been monitored also using both CV and the out-of-phase currents response

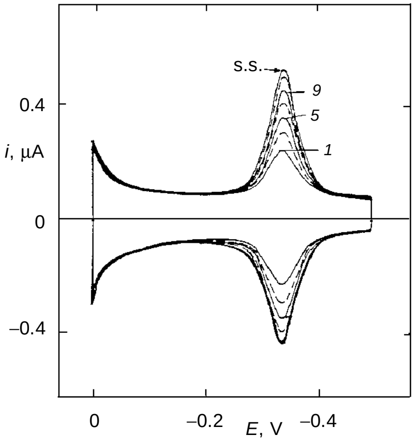


FIG. 1
Repetitive cyclic voltammograms for 5 . 10^{-6} mol l^{-1} NR in universal buffer solution pH 3.2. Start at E_s = 0.0 V, scan rate 100 mV s^{-1}; *1*, *5*, *9* numbers of the cycle, s.s. is for steady state cycle

to an AC signal in the pH range of 7.2 – 10.2 (Fig. 3). The AC response shows a sharp peak split due to the pseudocapacity of the adsorbed electroactive species. At both sides of the peak the capacitive current is lower than that of the supporting electrolyte, indicating the onset of an adsorption of both oxidized and reduced forms of NR. The depression of the capacitive AC current in presence of NR reflects the adsorption region where the molecules of NR are adsorbed flat on the electrode surface[11–15].

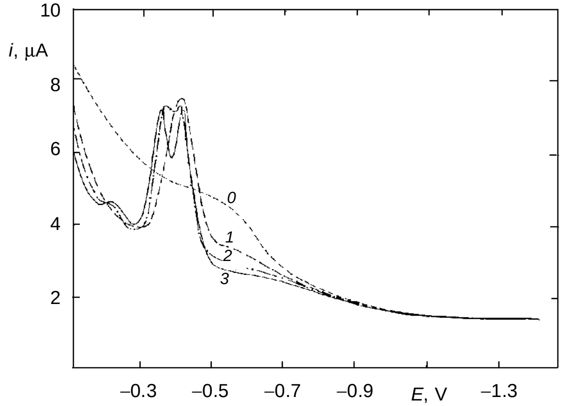


FIG. 2
AC voltammetric curves for NR at pH 3.2, scan rate 5 mV s^{-1}, amplitude 10 mV_{pp}, phase angle 90°, frequency 330 Hz and $t_s = 120$ s. *0* Backround, *1* 1.0 . 10^{-6}, *2* 2.0 . 10^{-6}, *3* 3.0 . 10^{-6} mol l^{-1} NR

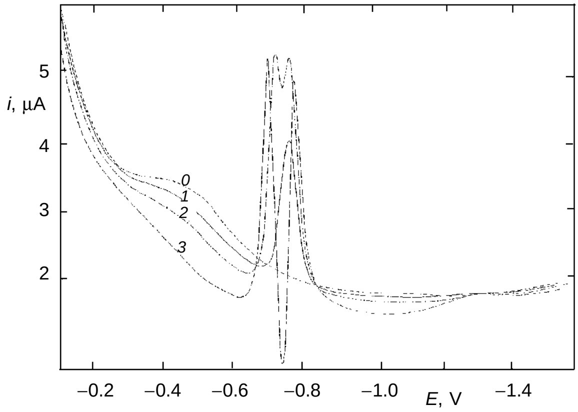


FIG. 3
AC voltammetric curves for NR at pH 9.2. *0* Backround, *1* 1.0 . 10^{-6}, *2* 2.0 . 10^{-6} , *3* 3.0 . 10^{-6} mol l^{-1} NR. Other conditions as in Fig. 2

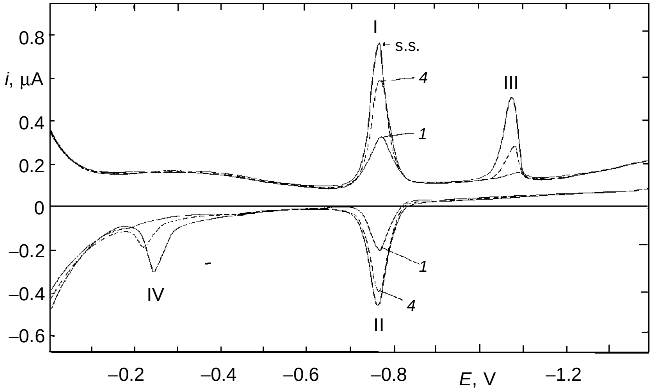


FIG. 4
Repetitive cyclic voltammograms for 5 . 10^{-6} mol l^{-1} NR at pH 9.2. Other conditions as in Fig. 1. Peaks I – IV are explained in text

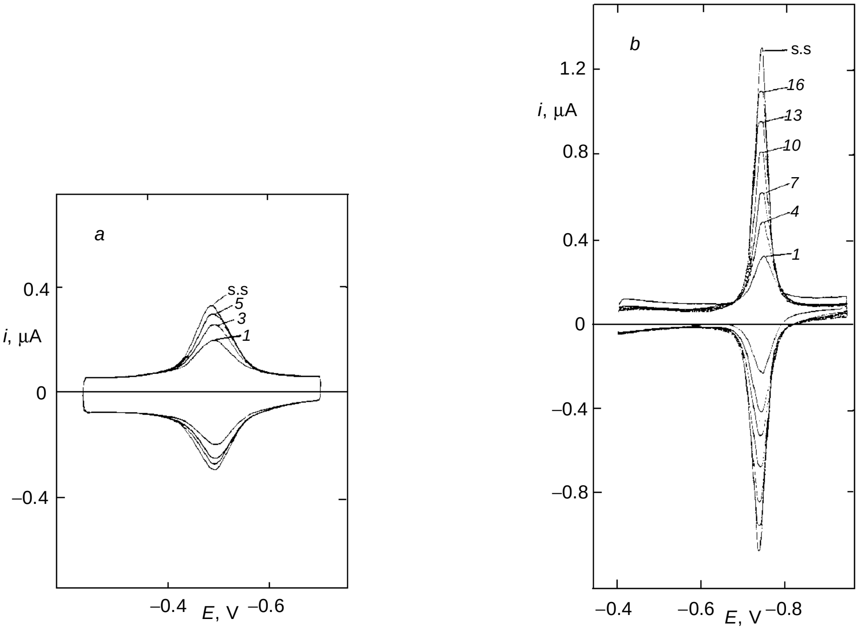


FIG. 5
Repetitive cyclic voltammograms (*1* – *19*) for 5 . 10^{-6} mol l^{-1} NR at pH 5.2 (*a*) and 9.2 (*b*). Other conditions as in Fig. 1

The CV of the adsorbed neutral NR species recorded at pH 9.2 is represented in Fig. 4. During the first few cycles a peak couple I, II appeared. Both peaks increased in magnitude and the peak potential shifted to less negative values. As pH increases (pH > 7.2) the half-peak width of both redox peaks decreases (Fig. 5). These results confirm that at pH > pK_a the repulsive forces between the adsorbed species are diminished and consequently strong adsorption and accumulation of the NR species are more favourable.

The results of CV indicate that the adsorbed amount of NR is proportional to the total charge Q consumed during the reduction of the adsorbed molecules, i.e. to the integral of the reduction current over the potential range of reduction E_s, E_e according to the equation

$$Q = \int_{E_s}^{E_e} i_s \mathrm{d}E = n\boldsymbol{F}A\Gamma \quad , \tag{1}$$

where n is the number of electrons in the electrode reaction, $\boldsymbol{F}$ the Faraday constant, A the surface area of the electrode and Γ the surface concentration of the adsorbed

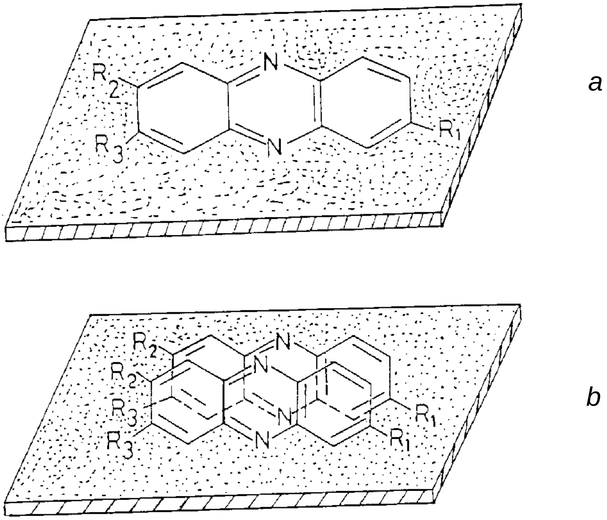
TABLE I

CV data of NR adsorbed from 5 . 10^{-6} mol^{-1} solution at varying pH values. Steady state scan on the mercury electrode surface, peaks I, II, half-peak width $\Delta E_{p/2}$, peak potential E_p

pH	Peak	Q_d, μC cm^{-2}	$\Delta E_{p/2}$, mV	$-E_p$, mV	i_p, μA
3.2	I	24.37	60.0	337.5	0.30
	II	20.04	62.5	337.5	0.25
4.2	I	25.33	60.1	400.0	0.35
	II	21.31	68.7	400.0	0.30
5.2	I	26.25	62.5	487.5	0.30
	II	20.00	75.5	487.5	0.25
6.2	I	33.33	68.70	537.5	0.40
	II	28.33	75.00	537.5	0.32
7.2	I	40.62	46.80	600.0	0.62
	II	33.75	50.00	600.0	0.55
8.2	I	60.00	37.50	637.5	0.95
	II	51.87	46.80	637.5	0.85
9.2	I	67.16	34.30	737.5	1.35
	II	59.58	37.56	737.5	1.15
10.2	I	70.72	35.31	750.0	1.20
	II	62.50	36.21	750.0	1.25
11.2	I	73.12	35.0	812.0	1.25
	II	70.42	35.0	812.5	1.25

species. Table I shows the values of the surface charge density Q_d for various pH values. The value of charge density Q_d = 24.37 μC cm^{-2} at pH 3.2 is similar to that calculated from LSV (ref.[10]) assuming formation of monolayer with orientation of phenazine system parallel to the electrode surface. On the other hand, the values of charge densities in the pH range of 7.2 – 10.2 clarify that the adsorbed neutral species of NR are able to form two or three layers rather than a monolayer of the protonated species (Schemes 2*a*, 2*b*). Moreover, NR species in neutral and alkaline solutions can form dimers or tetramers[10] which can be adsorbed on the electrode surface.

In neutral and alkaline solutions the CV of adsorbed NR species shows additional peak couple III, IV at potentials both positive and negative from the main redox couple I, II (Fig. 4). Appearance of the peak III is accompanied by 70% decrease of the charge density of peaks I, II indicating the desorption process at the potential of peak III. Holding the potential between I and III for ca 2 min leads to complete disappearance of peak II (Fig. 6). The AC response shows the same phenomena for the desorption process and a small capacitive peak at −1.1 V (pH 6.2) and at −1.3 V (pH 9.2, Fig. 3). On the other hand, peak IV at −0.225 V is probably a capacitive peak reflecting the surface rearrangement of the adsorption layer.



$R_1 = N(CH_3)_2$, $R_2 = CH_3$, $R_3 = NH_2$

SCHEME 2

In alkaline solutions at pH ≥ 8.5 ca 99.8% of the NR dye is in its neutral form D/DH_2 and consequently undergoes strong adsorption. The charge density Q_d vs the bulk concentration indicate an adsorption isotherm with an abrupt increase of surface coverage when the bulk concentration exceeds a critical value (Fig. 7).

It conreflects the formation of two- and threelayers by adsorption. In acidic buffer solutions, however, the adsorption isotherm shows a constant value at Q_d equal to ca 25 μC cm^{-2} which corresponds to monolayer only. In acidic solutions association and the aggregation of protonated NR species is not possible, hence formation of multilayers does not take place.

Stripping Voltammetry of Adsorbed NR

DP and AC voltammetry were used for the stripping analysis of adsorbed NR. Enhancement factors of 1.5 and 15 were calculated for DP and AC voltammetry, respectively, by comparing the peak intensities obtained after 2 min of preconcentration in $1 . 10^{-6}$ M NR. Therefore, an AC voltammetry was used to study the cathodic stripping behaviour of NR.

The AC stripping voltammetry of adsorbed NR was performed at concentration of $1 . 10^{-8}$ M NR in a universal buffer covering the pH range 3.2 – 11.2. At pH 9.2 NR exhibits a cathodic peak at –0.7 V corresponding to its reduction. The best developed peak for stripping analysis of NR was found at pH 9.2. At pH below 9.2 the peak height diminished over the whole pH range.

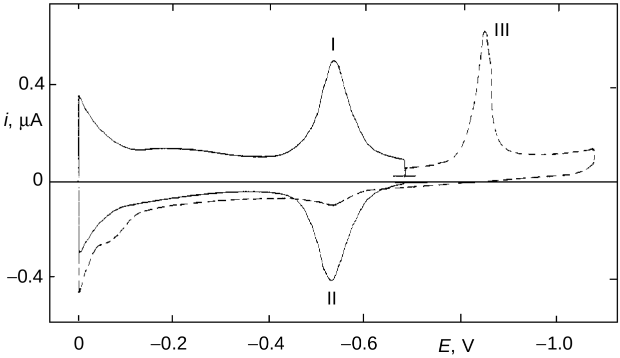


FIG. 6

Cyclic voltammograms of $5 . 10^{-6}$ mol l^{-1} NR at pH 6.2. The dashed line shows the influence of the holding potential between peaks I and III for ca 2 min. Other conditions are as in Fig. 1

The dependence of the adsorptive stripping peak current on the accumulation time at two different concentrations of NR is shown in Fig. 8. Current–time profiles at various concentrations represent corresponding adsorption isotherms. As in all types of stripping techniques, the choice of accumulation time requires a trade off between sensitivity and speed. Accumulation times in the range of 2 – 5 min are sufficient for quantitative determination of NR at 10^{-7} – 10^{-9} mol l^{-1} concentration levels.

The effect of the accumulation potential on the AC stripping current was examined at the optimum pH 9.2. At accumulation potentials more negative than 0.0 V, the peak height changed markedly with the accumulation potential. The optimum accumulation potential was found to be 0.0 V. This accumulation potential was therefore adopted for the electroanalytical determination of NR.

The AC stripping voltammograms of NR at various scan rates at pH 9.2 were recorded. At higher scan rates ($\upsilon > 20$ mV s^{-1}) the peak width increases, whereas at slower scan rates ($\upsilon < 20$ mV s^{-1}) the peak height decreases. Therefore, a scan rate of 20 mV s^{-1} was used for the optimum resolution.

From the above-mentioned results the optimum conditions for the analytical application of stripping voltammetry of NR were found: pH 9.2, scan rate 20 mV s^{-1}, accumulation time 5 min, accumulation potential 0.0 V and frequency 400 Hz. Under these

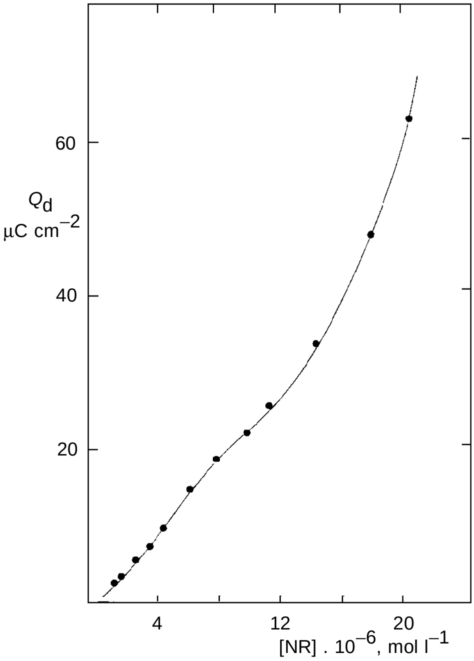


FIG. 7
Adsorption isotherm of NR in universal buffer solution, pH 9.2

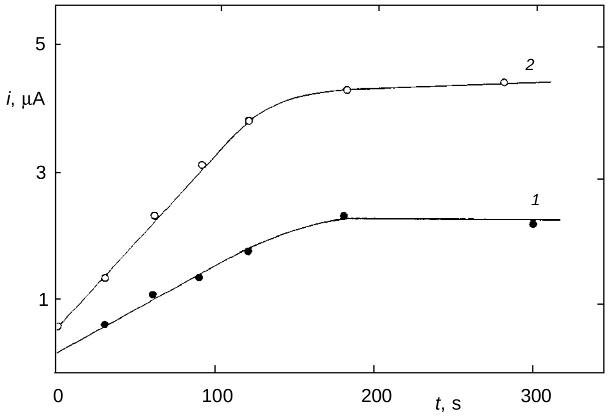


FIG. 8
Effect of the preconcentration time on the AC stripping current for *1* 4.76 . 10^{-8} and *2* 9.09 . 10^{-7} mol l^{-1}. pH 9.2, scan rate 20 mV s^{-1}, E_s = 0.0 V

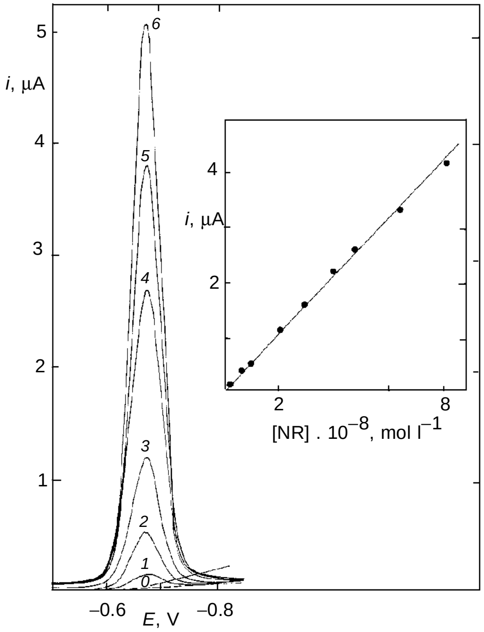


FIG. 9
AC stripping voltammograms for solutions of increasing NR concentration. *0* Backround, *1* 1.99 . 10^{-9}, *2* 9.9 . 10^{-9}, *3* 1.96 . 10^{-8}, *4* 4.76 . 10^{-8}, *5* 8.25 . 10^{-8}, *6* 1.07 . 10^{-7} mol l^{-1}, pH 9.2, preconcentration for 5 min, E_s = 0.0 V, scan rate 20 mV s^{-1} and frequency 400 Hz (*a*); the resulting calibration plot (*b*)

conditions, the calibration graph was linear up to 1 . 10^{-7} mol l^{-1} (Fig. 9). The data from set of 3 – 5 repeated measurements were subject to the least square refinement. The calibration curve generated over the concentration range of 1 . 10^{-9} – 3 . 10^{-7} mol l^{-1} had a slope of 5.14 ± 0.57 μA and the regression coefficient of 0.995. The detection limit was 1.0 . 10^{-9} mol l^{-1} NR.

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