THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 2,6-DICHLORO-4-NITRO-2'-ACETYLAMINO-4'--DIETHYLAMINOAZOBENZENE*

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The polarographic reduction of the title azo dye has been studied, a mechanism has been proposed and optimal conditions have been found for its analytical use. The detection limit using a classical dropping mercury electrode was $2 . 10^{-7}$ mol l^{-1} for TAST polarography and $1 . 10^{-8}$ mol l^{-1} for differential pulse polarography. Using a hanging mercury drop electrode, the detection limit was $9 . 10^{-9}$ mol l^{-1} for fast scan differential pulse voltammetry and $1 . 10^{-8}$ mol l^{-1} for linear scan voltammetry. Adsorption accumulation of the test substance on the surface of the hanging mercury drop electrode led to a further decrease in the detection limit to $1 . 10^{-9}$ mol . $. l^{-1}$ for fast scan differential pulse voltammetry and $7 . 10^{-10}$ mol l^{-1} for linear scan voltammetry.

Azo dyes, which form the largest group of organic dyes, constitute more than 35% of the global production of all dyes and thus are encountered by human beings in their living, working and natural environment. A number of azo dyes exhibit genotoxic or ecotoxic properties[1,2] leading to the need for sensitive and selective methods for determining these substances. Because of the easy reducibility of the azogroup[3-6], polarographic methods have recently been used for the determination of azo dyes, especially in the more modern forms, such as differential pulse polarography (DPP), fast scan differential pulse voltammetry (FS DPV), linear scan voltammetry (LSV) and adsorptive stripping voltammetry, whose principles, capabilities and limitations have been described in monographs and reviews[7-9].

This work deals with the determination of 2,6-dichloro-4-nitro-2'-acetylamino-4'--diethylaminoazobenzene (formula *I* in Eq. (*A*)), which is an industrially manufactured azo dye, using modern polarographic and voltammetric methods. As this substance is poorly soluble in water, so far only its titanometric[10], spectrophotometric[11] and polarographic[12,13] determination in acetonitrile medium have been described.

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A mixed water–methanol medium was used in this work; this mixture ensures sufficient solubility of the test substance, is more readily available than anhydrous acetonitrile and has been found to be useful in the determination of poorly soluble azo dyes[14-16].

EXPERIMENTAL

A pure sample of the studied azo dye was obtained from the Research Institute for Organic Synthesis, Pardubice-Rybitví. The preparation of solutions of this substance, of the other reagents and the instruments used were the same as in ref.[15]. The pulse height in DPP was − 50 mV. A mercury reservoir height of $h = 81$ cm was used, yielding a DME flow rate of $m = 1{\cdot}34\ \mathrm{mg\ s^{-1}}$ and drop time of $\tau = 4{\cdot}21$ s (in 0·1 mol $\mathrm{l^{-1}}$ KCl at an applied potential of 0 V).

Procedures

The calibration curves were measured and treated as described in ref.[15].

The number of electrons exchanged was found using coulometry at constant potential with the following procedure: 50·0 ml of Britton–Robinson buffer, pH 6, and 40 ml methanol were measured into the coulometric vessel (final pH 7·0) and the solution was bubbled with nitrogen. Simultaneously, pre-electrolysis was initiated at a selected constant potential. After about 20 minutes, the residual current value decreased below 0·2 mA and did not change. The appropriate parameters for the automatic residual current compensation circuit were then adjusted and 10·00 ml of the studied azo dye in methanol were added ($c = 1 \cdot 10^{-4}$ mol $\mathrm{l^{-1}}$) which had also been prebubbled with nitrogen. The electrolysis was terminated after the current decreased to the residual current value (cca 45—60 min) and the charge consumed was found by digital integration of the current passed. The course of the reaction was studied by TAST polarography and spectrophotometry by removing 10 ml of the solution at given time intervals and measuring its TAST polarographic curve or spectrum in the visible and UV regions. The course of the reaction was studied by thin-layer chromatography by extracting the solution obtained after completion of the electrolysis by 2 × 20 ml benzene and evaporating the combined extracts under reduced pressure to dryness on a rotating vacuum evaporator; the residue was dissolved in 1 ml of methanol. The solution formed (30 μl) was applied to the start of a Silufol UV 254 thin layer (Kavalier, Votice) and was chromatographed by the ascending technique using a benzene–ethylacetate (5 : 1) mobile phase in an atmosphere saturated by the mobile phase vapours. In addition to visual detection of the coloured substances, the colourless products were detected on the basis of extinction of the fluorescence of the background after irradiation with UV radiation with a wavelength of 254 nm and also on the basis of their colour reaction with *p*-dimethylaminobenzaldehyde after spraying the chromatogram with a 1% solution of this substance in an ethanol–conc. hydrochloric acid mixture (95 : 5).

RESULTS AND DISCUSSION

First the stability of the stock solution of the studied azo dye in methanol ($c = 1 \cdot 10^{-4}$ mol $\mathrm{l^{-1}}$) was studied spectrophotometrically. No change occurred in the absorbance ($\varepsilon = 2{\cdot}95 \cdot 10^4\ \mathrm{mol^{-1} \cdot l\ cm^{-1}}$) (see Fig. 1) at the wavelength of the absorption maximum ($\eta = 488$ nm) over a period of 30 days.

TAST *and* DP *Polarography at a Dropping Mercury Electrode*

Table I depicts the effect of the pH on the TAST and DP polarograms of the studied azo dye. Two waves or peaks appeared on the polarograms at all the pH values (see Fig. 2), connected with the presence of two electroactive groups in the molecule (—NO_2 and —N=N—). The heights of the two waves in the TAST polarogram are comparable, indicating that the same number of electrons is exchanged in both cases. The observed shift of the wave or peak to more negative potentials with increasing pH can be described by the relationships given in Table II, whose parameters were calculated by the method of linear regression. This shift can be explained on the basis of protonation of the molecule of the studied azo dye, leading to a decrease

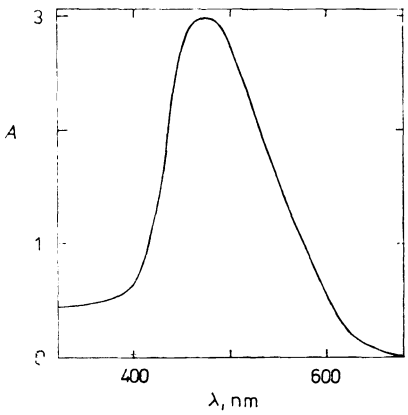


FIG. 1

The visible spectrum of a solution of the studied azo dye in methanol ($c = 1 . . 10^{-4}$ mol l^{-1}) in a cuvette with pathlength of 1 cm

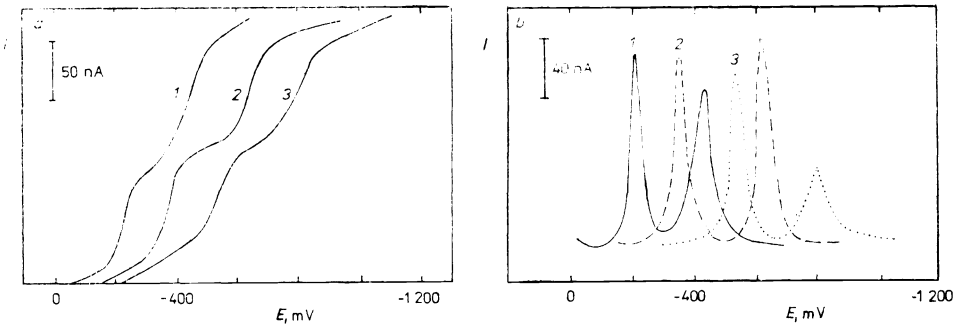


FIG. 2

The effect of the pH on the TAST (*a*) and DP (*b*) polarograms of the studied azo dye ($c = 1 . . 10^{-5}$ mol l^{-1}) in mixed Britton–Robinson buffer–methanol medium (1 : 1). pH: *1* 4·03, *2* 7·04, *3* 10·34

in the electron density on the electroactive functional groups, facilitating acceptance of an electron during the polarographic reduction. The height of the TAST wave is practically pH-independent, indicating that the number of electrons exchanged does not change over the whole studied pH range. The changes in the height of the DPP peaks reflect changes in the reversibility of the studied processes, appearing as changes in the slope of the logarithmic analysis. The best developed wave or peak for analytical utilization was obtained in a medium with pH 7·0 (see Fig. 2), in which all the subsequent dependences were studied. In this medium, the heights of both waves are diffusion controlled, confirmed by TAST polarography in the constant value of the expression $I_{lim}/m^{2/3}\tau^{1/6}$. For m in the range 0·8–1·5 mg s^{-1} and τ

TABLE I

The effect of the pH on the TAST and DP polarograms of the test azo dye[a]

pH	$E_{1/2}$ mV	I_{lim} nA	E_p mV	I_p nA	Slope[b] mV
2·77	−150[c]	48	−145	121	28·1
	−355[c]	69	−345	90	57·2
4·03	−220	50	−225	128	33·6
	−450	68	−450	107	56·5
4·87	−265	50	−255	129	30·2
	−515	55	−510	110	43·6
5·88	−305	63	−305	130	35·7
	−580	59	−580	122	44·3
7·04	−360	65	−360	124	40·4
	−640	65	−635	139	38·6
8·07	−400	66	−405	115	45·1
	−685	65	−680	132	38·6
8·82	−425	65	−435	96	49·6
	−695	62	−695	104	44·5
9·31	−470	65	−490	87	63·1
	−750	61	−745	63	57·0
10·34	−530	61	−545	102	48·2
	−800	60	−795	49	72·1
11·08	−585	61	−585	124	29·4
	−825	60	−810	46	62·5

[a] $c = 1 . 10^{-5}$ mol l^{-1}, Britton–Robinson buffer – methanol medium (1 : 1); [b] slope of the dependence of E on $\log (I_{lim} - I)/I$; [c] the upper value corresponds to the first wave or peak, the lower to the second wave or peak.

in the range 1–2 s, this expression has the value $47 \pm 1{\cdot}7\ \mu A\ g^{-2/3}\ s^{-1/6}$ for the first wave and $47 \pm 2\ \mu A\ g^{-2/3}\ s^{-1/6}$ for the second wave. The diffusion control of the studied process is also confirmed by the linear dependence of the wave height in TAST polarography or peak height in DPP on the concentration of the azo dye (see Table III).

It followed from the logarithmic analysis that neither the 1st nor the 2nd wave corresponds to a reversible process. This fact was further confirmed by cyclic voltammetry at a hanging mercury drop electrode (see Fig. 3). No anodic peak was observed on the cyclic voltammogram in the whole studied pH interval at polarization rates of $5-100\ mV\ s^{-1}$, even when the polarization direction was reversed in the region between the 1st and 2nd peak or in the region beyond the second peak. The fact that the height of the cathodic peak is not directly proportional to the square root of the polarization rate indicates that a simple irreversible charge transfer is not involved, but rather a more complex process that is also apparently complicated by adsorption of the studied substance on the working electrode surface.

It was found by constant potential coulometry at −800 mV that electrolysis in Britton–Robinson buffer – methanol medium (1 : 1) at pH 7 for 60 minutes corresponds to the exchange of 7·9 electrons per molecule of the studied substance. The charge passed after 45 minutes in this medium at a constant potential of −500 mV corresponded to the exchange of 3·8 electrons. If the constant potential was then shifted to −800 mV and the electrolysis continued for a further 45 minutes, then the total charge passed corresponded to the exchange of 8·1 electrons per molecule of

TABLE II

The parameters of the dependence $E = a + b$ pH for the half-wave potential in TAST polarography and the peak potential in DPP and FS DPV

Method	pH	1st wave or peak		2nd wave or peak	
		a, mV	b, mV pH^{-1}	a, mV	b, mV pH^{-1}
TAST	3–6	−14	−50·3	−155	−72·8
	6–8	−68	−40·9	−349	−40·3
	9–11	175	−68·5	−223	−55·6
DPP	3–6	−11	−50·6	−140	−75·5
	6–9	−46	−44·3	−349	−40·1
	9–11	114	−63·5	−272	−49·5
FS DPV	3–6	−3	−47·0	−149	−65·8
	6–9	−32	−41·0	−387	−30·0
	9–11	71	−52·5	−137	−55·8

the studied substance. It can thus be assumed that each of the observed waves corresponds to the exchange of 4 electrons under the given conditions.

It can be seen from the spectrophotometric study of the course of the reduction during constant potential coulometry at −800 mV (see Fig. 4*a*) that the absorption band at 492 nm disappears; this band corresponds to the azogroup. The absorbance in the region around 400 nm, where absorption by the nitrogroup could be expected, is also negligible. When reduction is carried out at a potential of −500 mV, the absorbance of the azogroup at 492 nm also decreases markedly (see Fig. 4*b*) but the absorbance around 400 nm, apparently connected with the nitrocompound formed, increases somewhat. This can be explained by a shift in the absorption maximum of the nitrogroup to shorter wavelengths as a result of reduction of the azogroup,

TABLE III

The parameters of the calibration straight lines and detection limits for various methods of determining the test azo dye in mixed methanol – Britton–Robinson buffer medium (1 : 1), pH 7·13

Method Wave(peak)	c μmol l^{-1}	b^a mA mol^{-1} l	a^a nA	r^a	$L_D{}^a$ μmol l^{-1}
TAST					
1	1—10	5·9	0·5	0·9998	—
2	1—10	6·1	0·8	0·9998	—
1	0·1—1	6·0	0·1	0·9940	0·2
2	0·1—1	5·9	0·4	0·9913	0·3
DPP					
1	0·1—1	13·9	0·2	0·9998	—
2	0·1—1	14·2	0·5	0·9992	—
1	0·01—0·1	24·0	−0·2	0·9991	0·01
2	0·01—0·1	25·0	−0·2	0·9976	0·02
FS DPV					
1	0·1—1	35·9	7·1	0·9990	—
2	0·1—1	49·8	3·8	0·9987	—
2[b]	0·01—0·1	55·5	−0·2	0·9984	0·009
2[c]	0·001—0·01	940	0·5	0·9974	0·001
LSV					
1	0·1—1	55·3	2·0	0·9966	—
2	0·1—1	72·0	2·4	0·9989	—
1	0·01—0·1	48·0	1·0	0·9984	0·03
2	0·01—0·1	70·5	0·2	0·9966	0·01
2[b,c]	0·001—0·01	1 320	−0·1	0·9991	0·0007

[a] *b* slope, *a* intercept, *r* correlation coefficient, L_D detection limit; [b] 1st peak cannot be evaluated [c] accumulation for 2 minutes in stirred solution; recorded 15 s after termination of stirring.

producing a bathochromic shift in the band of the nitrogroup as it permits more extensive conjugation of the double bonds. The gradual changes in the spectrum with time after completion of electrolysis (see Fig. 4c) with the existence of an isosbestic point indicate that the substances formed in the first stage of the reduction can also undergo subsequent chemical reaction.

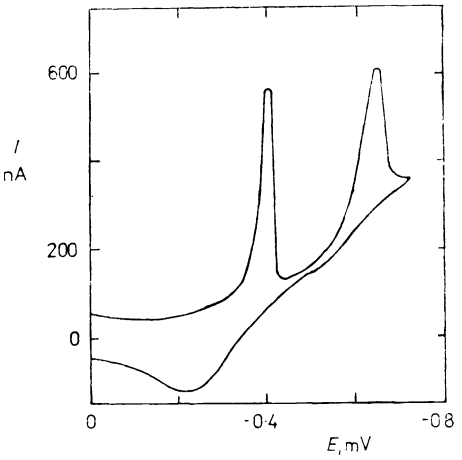


FIG. 3

The cyclic voltammogram of the studied azo dye ($c = 1 . 10^{-5}$ mol l^{-1}) in methanol–Britton–Robinson buffer medium (1 : 1), pH 7·0, with a polarization rate of 50 mV s^{-1}

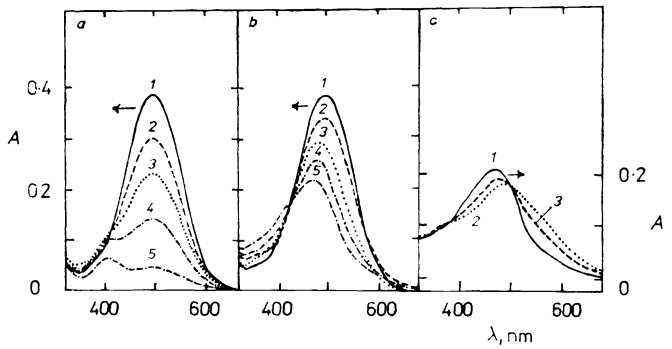


FIG. 4

Spectrophotometric study of the coulometric reduction of the studied azo dye ($c = 1 . 10^{-5}$ mol . $. l^{-1}$) in Britton-Robinson buffer – methanol medium (1 : 1), pH 7. *a* Spectra of the solution after passage of charge corresponding to $n = 0(1)$, $2(2)$, $4(3)$, $6(4)$, and $8(5)$ at a constant potential of −800 mV; *b* spectra of the solution after passage of charge corresponding to $n = 0(1)$, $1(2)$, $2(3)$, $3(4)$, and $4(5)$ at constant potential of −500 mV; *c* spectra of the solution after passage of charge corresponding to $n = 4$ at −500 mV, measured $2(1)$, $4(2)$, and $6(3)$ minutes after completion of electrolysis

It followed from the TAST polarographic study of the coulometric reduction of the test substance that, when the reduction is carried out at −500 mV, the height of the 1st wave decreases, while that of the 2nd wave is practically unchanged. The dependence of the height of the 1st wave on the charge passed, recalculated to the number of electrons n per molecule of studied substance is linear and intercepts the abscissa at a value of $n = 4{\cdot}3$. When the reduction is carried out at a constant potential of −800 mV, the heights of both waves decrease simultaneously, the dependence on n is again linear, with an intercept of $n = 8{\cdot}3$ for the first wave and 8·4 for the second wave.

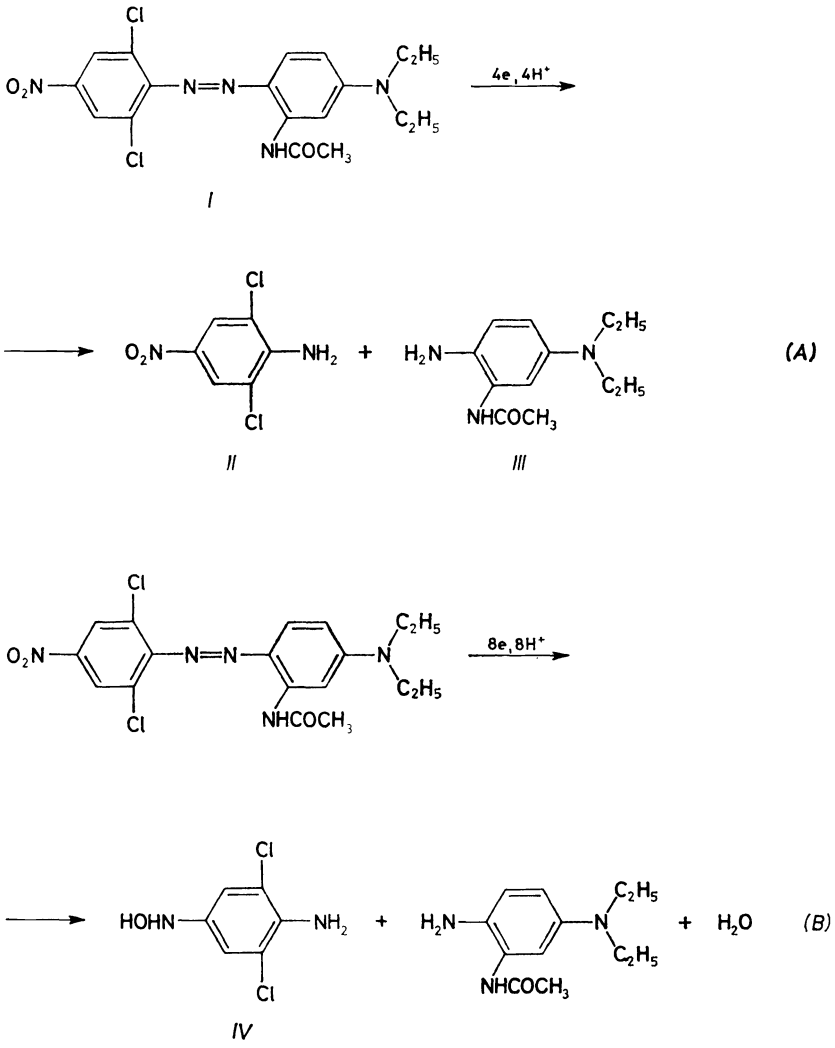
Study of the products of the coulometric reduction at −500 mV using thin-layer chromatography with the procedure given under experimental with visual chromatogram evaluation yielded three spots with R_F 0·27 (light yellow), 0·43 (purple, traces) and 0·64 (red, traces, corresponding to the original azodye). Irradiation of the chromatogram with UV radiation with a wavelength of 254 nm led to emphasis of the spot at $R_F = 0{\cdot}27$ on the basis of extinction of the fluorescence of the background and a further spot was observed at $R_F = 0{\cdot}74$ corresponding to a colourless substance. Spraying of the chromatogram with a solution of *p*-dimethylaminobenzaldehyde, yielding a yellow colour with aromatic amines, led to marked emphasis of the yellow colour of the spot at $R_F = 0{\cdot}27$ and to yellow coloration of the previously colourless spot at $R_F = 0{\cdot}74$. It can be assumed on the basis of these observations that the yellow spot at $R_F = 0{\cdot}27$ corresponds to an aromatic nitrocompound containing an amino group (see substance *II*, Eq. (*A*)) and that the colourless spot at $R_F = 0{\cdot}74$ corresponds to an aromatic amine (see substance *III*, Eq. (*A*)). If the solution after the reduction is left to stand for 12 h, then the chromatogram contains a spot at $R_F = 0{\cdot}43$, apparently corresponding to subsequent reaction of the products of reductive splitting of the studied azodye. Coulometric reduction at −800 mV leads to complete disappearance of the yellow colour corresponding to the spot at $R_F = 0{\cdot}27$ and a spot can be observed at $R_F = 0{\cdot}25$, visible under a UV lamp or after spraying with *p*-dimethylaminobenzaldehyde, apparently corresponding to the aromatic amine (see substance *IV*, Eq. (*B*)).

It can be assumed on the basis of the above facts that the first wave corresponds to the 4-electron, irreversible reduction of the azogroup according to Eq. (*A*), while the second wave corresponds to the 4-electron, irreversible reduction of the nitrogroup according to Eq. (*B*).

This assumption is also in agreement with the mechanism of the reduction of *p*-nitroazobenzene[17], where the reduction of the azogroup also preceeds the reduction of the nitrogroup.

It followed from study of the stability of variously concentrated solutions of the studied azodye ($c = 1 \cdot 10^{-5}$ to $1 \cdot 10^{-7}$ mol l^{-1}) in the polarographed medium (i.e. Britton–Robinson buffer – methanol (1 : 1), pH 7) that measurements should be

carried out 10 minutes after solution preparation. At times greater than 20 minutes, a decrease in the peak height can be observed, attaining approximately 5% of the original peak height after 30 minutes for a $1 . 10^{-6}$ mol l^{-1} solution and 10% for a $1 . 10^{-7}$ mol l^{-1} solution.



The parameters of the calibration straight lines and the calculated values of the detection limit are given in Table III, from which it can be seen that determination using the DME is more sensitive when the more positive peak is employed, corresponding to the reduction of the azo group.

TABLE IV

The effect of the pH on the position (E_p) and height (I_p) of the peak of the studied azo dye in FS DPV at the HMDE

pH	E_p^a mV	I_p^a nA	E_p^b mV	I_p^b nA
2·66	−125	19·6	−320	30·6
3·94	−190	24·7	−410	28·2
4·89	−240	31·4	−480	35·1
5·90	−275	34·6	−530	39·5
7·13	−325	36·3	−595	48·5
8·29	−370	31·1	−625	46·5
8·82	−395	28·2	−645	44·7
9·33	−415	26·2	−660	39·8
9·99	−460	25·0	−695	23·2
10·80	−490	30·3	−735	15·3
11·82	−550	32·7	−800	23·2

[a] 1st peak; [b] 2nd peak; $c = 1 \cdot 10^{-6}$ mol l^{-1} in mixed Britton–Robinson buffer – methanol medium (1 : 1).

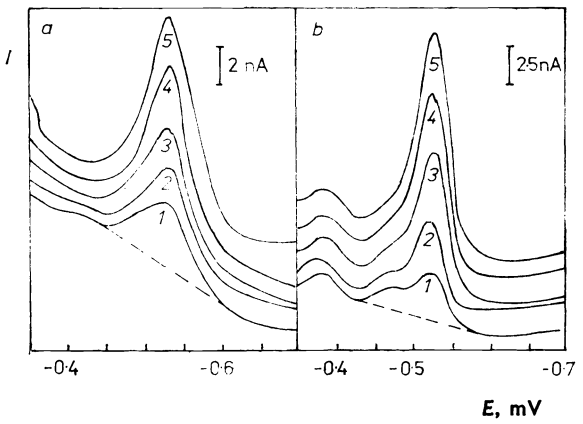


FIG. 5

FS DPV (*a*) and LSV (*b*) recordings at the HMDE after 2 minute adsorptive accumulation in stirred solution. Concentration of the studied azo dye (nmol l^{-1}): *1* 2; *2* 4; *3* 6; *4* 8; *5* 10. The dashed line corresponds to the baseline from which the peak height was measured

Fast Scan Differential Pulse Voltammetry and Linear Scan Voltammetry at a Hanging Mercury Drop Electrode

Table IV gives the effect of the pH on the positions and heights of both peaks in FS DPV at the HMDE. Table II lists the parameters of the dependence of E_p on the pH calculated by the least squares method. This dependence has the same character as for TAST or DP polarography and can be explained analogously. The dependence of the peak height on the pH reflects changes in the reversibility of electrode process, which also appear in the value of the slope of the logarithmic analysis.

For analytical purposes, the best developed and highest peaks are those obtained at pH 7·13. Therefore, this medium was employed to measure all the subsequent dependences. The dependence of the height of the FS DPV peak on the concentration of the azodye is linear in this medium in the range $10^{-6}-10^{-7}$ mol l^{-1} for the 1st peak and $10^{-6}-10^{-8}$ mol l^{-1} for the 2nd peak (see Table III). It was further found that the peak height depends on the time elapsed between formation of the mercury drop and recording of the voltammogram, which can be explained by adsorptive accumulation of the studied substance on the surface of the working electrode. The measurement of the effect of a number of parameters (methanol content, buffer concentration, stirring, time, pH) led to use of 2-minute accumulation in stirred solution in a 10-fold diluted Britton–Robinson buffer – methanol mixture (1 : 1) with pH 7·2. The second peak was evaluated and its height was measured relative to the straight line connecting the minima on both sides. The first peak was much smaller under these conditions.

A further increase in the sensitivity and decrease in the noise together with increased determination rate can be attained by using LSV, where well-developed peaks were obtained using polarization rates of up to 50 mV s^{-1}. Measurement of the effect of the pH on the positions and heights of both peaks revealed that these dependences have the same character as for FS DPV and that the optimal conditions for LSV determination are identical to those for FS DPV.

For illustration, Fig. 5 depicts the FS DP voltammograms and LS voltammograms for the lowest useful concentration range. The parameters of the calibration straight lines and the calculated detection limits are given in Table III.

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