

THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION
OF 2-AMINO-5-SULPHOAMOYLNAPHTHALENE-AZO-(2'-CHLORO-
-4'-NITROBENZENE)*

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The polarographic reduction of the title azo dye was studied, a mechanism was suggested and optimal conditions were determined for analytical utilization of this process. A detection limit of $3 \cdot 10^{-7} \text{ mol l}^{-1}$ was obtained using fast scan differential pulse voltammetry and $0.7 \cdot 10^{-7} \text{ mol l}^{-1}$ using linear potential scan voltammetry at a hanging mercury drop electrode. The detection limit can be decreased by adsorptive accumulation of the determined substance on the surface of the hanging mercury drop electrode, to $1 \cdot 10^{-8} \text{ mol l}^{-1}$ for fast scan differential pulse voltammetry and $0.6 \cdot 10^{-8} \text{ mol l}^{-1}$ for linear scan voltammetry. The selectivity of the determination can be improved by preliminary separation by extraction or thin-layer chromatography.

The study of the polarographic behaviour of azo dyes is interesting from several points of view. Primarily, polarography can be employed to determine the contents of these substances in industrial products. Further, modern modifications of polarographic methods can be used to monitor low concentrations of these substances in the environment around industrial areas. This aspect is especially important in connection with the ecotoxicological properties of a number of azo compounds. Finally, the mechanism of the polarographic reduction can form a basis for the study of metabolic transformations of these substances *in vivo*. In the framework of a systematic study of the polarographic behaviour of dyes, this work was devoted to 2-amino-5-sulphoamoylnaphthalene-azo-(2'-chloro-4'-nitrobenzene) (Formula I, Eq. (A)), an industrially manufactured azopigment. Because of the limited solubility of this substance in water, its titanometric¹, spectrophotometric², and polarographic^{3,4} determinations have so far been studied in acetonitrile medium. The polarographic behaviour of some other derivatives of naphthaleneazobenzene has been studied in aqueous, mixed, and nonaqueous media. The results of these studies have been reviewed, for example, in the monographs⁵⁻⁸. In this work, a mixed water-methanol medium was used, ensuring sufficient solubility of the studied substance, which is

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easier to employ than nonaqueous acetonitrile. The basic study was carried out using TAST polarography and differential pulse polarography (DPP) at a dropping mercury electrode (DME), the reversibility was studied using cyclic voltammetry (CVA) and the number of electrons exchanged was determined by potentiostatic coulometry. In an attempt to further increase the sensitivity of the polarographic determination of the studied azo dye, fast scan differential pulse voltammetry (FSDPV) and linear scan voltammetry (LSV) at a hanging mercury drop electrode (HMDE) and their combination with adsorptive accumulation on the surface of the working electrode were also employed. In conclusion, the potentialities of prior extraction separation or separation using thin-layer chromatography for improving the selectivity were studied.

EXPERIMENTAL

Reagents

The stock solution of the studied azo dye ($c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$) was prepared by dissolving 0.04055 g of the pure substance (Research Institute for Organic Synthesis, Pardubice-Rybitví) in 1 l of p.a. methanol. More dilute solutions were prepared by exact dilution of the stock solution with methanol. All the solutions were stored in the dark. The content and purity of the substance were controlled titanometrically¹ and using thin-layer chromatography². Britton-Robinson buffer solutions were prepared in the usual manner⁹. The actual pH value in the methanol-buffer mixture (1 : 1) was determined using a combined glass and calomel GK 2320c electrode (Radiometer, Copenhagen), calibrated using acetate, borate, and phosphate buffers in 50% (v/v) methanol^{10,11}. The remaining chemicals and solvents were p.a. purity (Lachema, Brno). The water employed was doubly distilled in a quartz apparatus.

Apparatus

Polarographic and voltammetric measurements were carried out using a PA 3 analyzer with an XY-4105 recorder (Laboratorní přístroje, Prague) in a three-electrode arrangement with a saturated calomel electrode (SCE) and platinum wire auxiliary electrode. Thus, all the potential values are referred to the SCE. Where not stated otherwise, measurements using the DME (i.e. DC, TAST, DP, and AC techniques) were carried out at a polarization rate of 5 mV s^{-1} , controlled drop time of 1 s, mercury reservoir height of 36 cm, and DPP pulse amplitude of -100 mV . The DME employed had the following parameters: at $h = 36 \text{ cm}$, $m = 0.81 \text{ mg s}^{-1}$, and $\tau = 10.37 \text{ s}$ in $0.1 \text{ mol l}^{-1} \text{ KCl}$ at a potential of 0 V. The working electrode for LSV, CVA, and FSDPV was a static mercury drop electrode (SMDE) with a capillary diameter of 0.136 mm (Laboratorní přístroje, Prague), connected as an HMDE. Where not stated otherwise, the measurements were carried out at a polarization rate of 20 mV s^{-1} at the maximal drop size obtained by opening the valve for 160 ms.

Oxygen was removed from the analyzed solution by bubbling the solution for 10 min with nitrogen, which was purified by passing through an alkaline solution of sodium anthraquinone-2-sulphonate and solutions of chromium(II) ions in dilute hydrochloric acid over zinc amalgam. A pre-bubbler containing a 1 : 1 methanol-water mixture was placed prior to the polarographic vessel.

Coulometric measurements were carried out using an OH 404 coulometric analyzer (Radelkis, Budapest) in a 200 ml vessel, with the anode and cathode spaces separated by a glass frit; the cathode was a mercury pool at the bottom of the vessel. A saturated calomel reference electrode and platinum foil auxiliary electrode were employed. The solution was stirred using a magnetic stirrer during the determination and protected by an inert atmosphere of nitrogen which was continuously passed over the surface of the solution.

Spectrophotometric measurements were carried out on a Unicam SP-800 instrument (Cambridge, U.K.) in quartz cuvettes with a specific path length of 0.5 and 1 cm.

All the measurements were carried out at laboratory temperature.

Procedure

The calibration curves were measured in triplicate and evaluated by the linear regression method. The detection limit was calculated by the Skogerboe and Grant method¹² as the value $t \cdot s/a$, where s is the standard deviation of the experimental points from the calibration straight line calculated by the linear regression method, a is the slope of this straight line and t is the Student coefficient at the 99% confidence level, dependent on the number of points used in construction of the calibration curve.

Coulometry at constant potential was carried out by measuring 50.00 ml of the base electrolyte into the coulometric vessel and bubbling with nitrogen. Simultaneously, pre-electrolysis was commenced at the previously selected constant potential. After about 20 min, the residual current value decreased below 0.2 mA and remained constant. Then the appropriate circuit parameters were adjusted for automatic compensation of the residual current and 50.00 ml of a $1 \cdot 10^{-4}$ mol \cdot l^{-1} solution of the studied substance in methanol that had been pre-bubbled with nitrogen was added with constant stirring and bubbling with nitrogen. Electrolysis was terminated after the current decreased to the residual value. The reduction required 50 to 60 min and the charge consumed was found by digital integration of the current passed. The reduction was studied spectrophotometrically and polarographically by removing 10.00 ml of solution at set intervals and measuring the TAST polarographic curve and the spectrum in the UV and visible regions. Sampling was carried out prior to initiation of the coulometric reduction and after reduction of 25, 50, 75, and 100% of the studied substance. (Calculated relative to the number of electrons exchanged at the constant potential employed).

In the measurement of the electrocapillary curves, the drop time was measured as an average for 10 drops. The surface tension σ was calculated from the measured drop time τ using the relationship $\Delta\sigma/\sigma = 0.973 \Delta\tau/\tau$, where σ and τ are the values for an aqueous solution of 0.1 mol \cdot l^{-1} NaNO_3 and $\Delta\sigma$ and $\Delta\tau$ are the changes in the given quantity on transition to the studied solution¹³.

Thin-layer chromatography was carried out on commercial Silufol UV 366 thin-layers (Kavalič, Votice) by the ascending method with elution using a chloroform-methanol mixture (95 : 5). A band of a calculated amount (10 to 125 μ l of $1 \cdot 10^{-4}$ mol l^{-1} solution) of the azo dye in methanol was applied to the thin layer.

After developing and drying, the chromatogram was cut so that the spot lay on a rectangle with dimensions of 1.5×5.0 cm, which was then cut to form a point and bent at the opposite end so that it could be hung with the upper end dipping in a dish with solvent. The spot was developed in a saturated methanol vapour atmosphere by washing directly into the polarographic vessel. After evaporation of the solvent by hot air, 10.00 ml of the base electrolyte were added (Britton-Robinson buffer-methanol (1 : 1), pH 2.59) and the solution was polarographed in the usual manner.

In the determination after extraction, 10.00 ml of the given solution containing 10–50 μg of the determined azo dye, with pH adjusted to a value of 7.0, was extracted six times with 10 ml of diethyl ether. The combined extracts were evaporated on a rotating evaporator in vacuo to dryness; the residue was dissolved in 10 ml of a Britton–Robinson buffer–methanol mixture (1 : 1) with pH 2.59 and analyzed using FSDPV or LSV at an HMDE.

RESULTS AND DISCUSSION

The Polarographic Behaviour of the Studied Azo Dye

The effect of the pH on the TAST and DP polarograms of the studied substance is described in Table I. At all the pH values employed, the polarograms exhibited two waves or peaks, connected with the presence of two electroactive groups in the molecule ($-\text{NO}_2$ and $-\text{N}=\text{N}-$). It follows from the relative heights of the two waves and from the determined number of exchanged electrons (see below) that the first wave corresponds to the reduction of the azo group and the second to the reduction of the nitro group. The shift in the half-wave potentials and peak potentials to more negative values with increasing pH is apparently connected with prior protonation of the molecule of the studied substance, leading to a decrease in the electron density at the reduced groups, facilitating electron transfer, so that the substance is more readily reducible in acid medium. The best developed curves were obtained in medium with pH 2.59, in which all the dependences were measured. In this medium, the heights of both waves are controlled by diffusion, reflected in a linear dependence on the square root of the mercury reservoir height. In agreement with this conclusion, the concentration dependence for DC and TAST polarography is linear and expression $I_{\text{lim}}/m^{2/3}\tau^{1/6}$ is constant for m in the range 0.5–2 mg s^{-1} and τ in the range 1–4 s for TAST polarography.

It followed from the logarithmic analysis that the process is not reversible. This was also confirmed by DPP with positive and negative pulse polarity on the basis of diagnostic criteria derived by Birke¹⁴ to differentiate between reversible, quasi-reversible, and irreversible processes. The dependence of the peak height and position on the height and polarity of the modulation pulses in acid, neutral, and alkaline media is described in Table II and a selected polarogram is given for illustration in Fig. 1. It follows that $|I_{\text{p,a}}/I_{\text{p,c}}| < 1$ and $E_{\text{p,c}} - E_{\text{p,a}} \approx 50$ mV. It follows from the diagnostic criteria derived in ref.¹⁴ that the process is irreversible. As the pH increases, the determined values approach those derived for quasireversible processes.

The reversibility of the electrode process was also studied by CVA. For the sake of illustration, Fig. 2 depicts cyclic voltammograms at two different pH values. It can be seen that an anodic peak is present and that the behaviour of the system approaches quasireversibility as the pH increases. Table III documents the effect of the polarization rate on the cyclic voltammogram in strongly alkaline media.

In spite of the presence of the anodic peak, the high $E_{p,a} - E_{p,c}$ value and low $I_{p,a}/I_{p,c}$ value prevent designation of the system as quasireversible; however, it should be borne in mind that the $I_{p,c}$ value is increased by adsorption of the studied

TABLE I

The effect of the pH on the TAST and DP polarograms of a solution of the azo dye ($c = 5 \cdot 10^{-5} \text{ mol l}^{-1}$) in Britton-Robinson buffer - methanol medium (1 : 1)

pH	$E_{1/2}$ mV	I_{lim} μA	E_p mV	I_p μA	slope ^a mV
2.17	-185 ^b	0.120	-140	0.255	41.0
	-395 ^c	0.210	-350	0.420	78.1
2.59	-200	0.135	-155	0.405	26.3
	-425	0.213	-385	0.620	61.5
3.37	-255	0.140	-200	0.400	35.0
	-500	0.225	-455	0.590	64.2
3.96	-280	0.123	-230	0.355	35.5
	-545	0.190	-500	0.470	51.0
5.14	-325	0.153	-275	0.425	40.5
	-610	0.188	-560	0.430	50.4
5.82	-365	0.157	-315	0.475	40.4
	-655	0.195	-605	0.450	48.5
6.99	-415	0.145	-375	0.400	51.3
	-690	0.185	-660	0.405	50.6
7.97	-460	0.155	-430	0.450	42.6
	-705	0.205	-670	0.505	44.4
8.73	-505	0.165	-470	0.435	50.4
	-740	0.190	-710	0.505	27.2
9.60 ^d	-550	0.153	-505	0.455	33.2
	-785	0.145	-750	0.255	34.9
	-1 250	0.021	-955	0.023	
10.16 ^d	-575	0.163	-530	0.490	32.1
	-825	0.125	-800	0.110	36.5
	-990	0.018	-960	0.017	
11.37	-610	0.163	-555	0.475	24.0
	-920	0.244	-865	0.275	97.5

^a The slope of the dependence of E vs $\log(I_{lim} - I)/I$; ^b the upper number always corresponds to the 1st wave or peak; ^c the lower number always corresponds to the 2nd wave or peak; ^d the 2nd wave or peak is split.

substance on the surface of the working electrode. This phenomenon can also explain the fact that the $I_{p,c}/v^{1/2}$ value is not constant, as required by the equations for completely irreversible processes¹⁵.

TABLE II

The effect of the pH on the position (E_p) and height (I_p) of the peak of a solution of the azo dye ($c = 5 \cdot 10^{-5} \text{ mol l}^{-1}$) for DPP with cathodic and anodic pulse polarity with a pulse height of 50 mV. Medium: Britton-Robinson buffer – methanol (1 : 1)

pH	$E_{p,c}^a$ mV	$I_{p,c}$ μA	$E_{p,a}^a$ mV	$I_{p,a}$ μA	$E_{p,c} - E_{p,a}$ mV	$ I_{p,a}/I_{p,c} $
2.59	-190 ^b	0.270	-240	0.105	50	0.39
	-417 ^c	0.340	-465	0.140	48	0.41
6.99	-405	0.290	-455	0.130	50	0.45
	-685	0.265	-730	0.140	45	0.53
10.16	-565	0.335	-620	0.125	55	0.37
	-815	0.070	-885	0.070	70	1.00

^a Indices a and c designate the anodic and cathodic pulse polarity, respectively; ^b the upper value always corresponds to the 1st peak; ^c the lower number always corresponds to the 2nd peak.

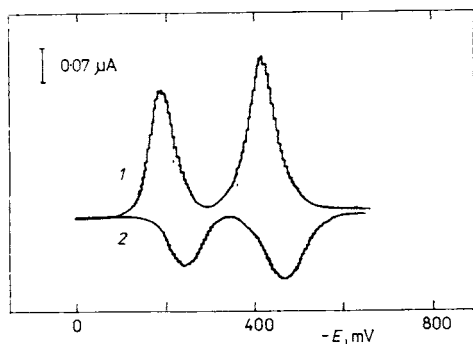


FIG. 1

The DP polarogram of the azo dye ($c = 5 \cdot 10^{-5} \text{ mol l}^{-1}$) in Britton-Robinson buffer – methanol medium (1 : 1) at pH 2.59 with cathodic (1) and anodic (2) pulse polarity with a pulse height of 50 mV

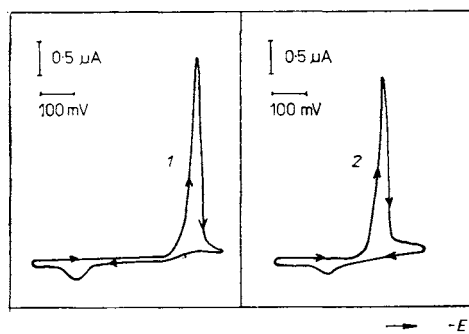


FIG. 2

Cyclic voltammograms of the azo dye ($c = 5 \cdot 10^{-5} \text{ mol l}^{-1}$) in Britton-Robinson buffer – methanol medium (1 : 1) with pH 7.27 (1) and 13.23 (2). Polarization rate 100 mV s^{-1} ; initial potential 0 V (1) and -0.35 V (2)

It was found by constant potential coulometry that reduction of the studied substance in acid medium at a potential of -300 mV involves exchange of 4 electrons and, at a potential of -750 mV, exchange of 10 electrons. These results are in agreement with the values obtained by TAST polarography, where the ratio of the heights of the two waves in acid medium is 2 : 3. If 60-minute reduction at -300 mV (determined value $n = 4$) in acid medium was followed by electrolysis at -750 mV, exchange of a further 4 electrons occurred over the next 60 minutes. If 60-minute reduction at -300 mV is followed by reduction at -1000 mV, a further 6 electrons are exchanged. In the alkaline region, 4 electrons are exchanged at a potential of -750 mV and 8 electrons at a potential of -1200 mV. These results correspond to the reduction of the $-\text{NO}_2$ group with exchange of 4 electrons only to the $-\text{NHOH}$

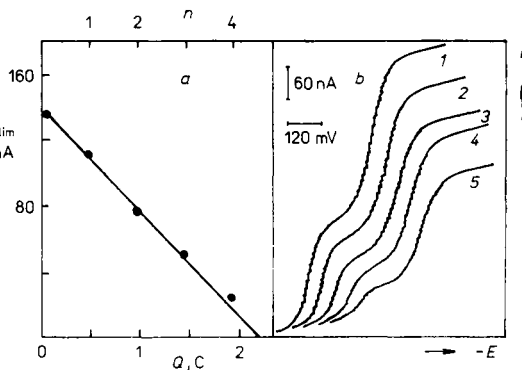
TABLE III

The effect of the polarization rate (v) on the position (E_p) and height (I_p) of the cathodic and anodic peaks in cyclic voltammetry. $c(\text{azo dye}) = 5 \cdot 10^{-1} \text{ mol l}^{-1}$, Britton-Robinson buffer-methanol (1 : 1), pH 13.49

v mV s $^{-1}$	$I_{p,a}^a$ μA	$I_{p,c}^a$ μA	$(I_{p,a}/I_{p,c})/v^{1/2}$ mV $^{-1/2}$ s $^{1/2}$	$I_{p,a}/v^{1/2}$ μA mV $^{-1/2}$ s $^{1/2}$	$I_{p,c}/v^{1/2}$ μA mV $^{-1/2}$ s $^{1/2}$	$E_{p,a}$ mV	$E_{p,c}$ mV	$E_{p,a} - E_{p,c}$ mV
10	— ^b	0.30	—	—	0.095	—	-665	—
20	— ^b	0.51	—	—	0.114	—	-675	—
50	0.09	0.98	0.013	0.013	0.138	-580	-690	110
100	0.14	1.42	0.009	0.014	0.142	-565	-700	135
200	0.17	1.56	0.008	0.012	0.110	-560	-720	160

^a Indices a and c designate anodic and cathodic peaks, respectively; ^b no anodic peak was observed.

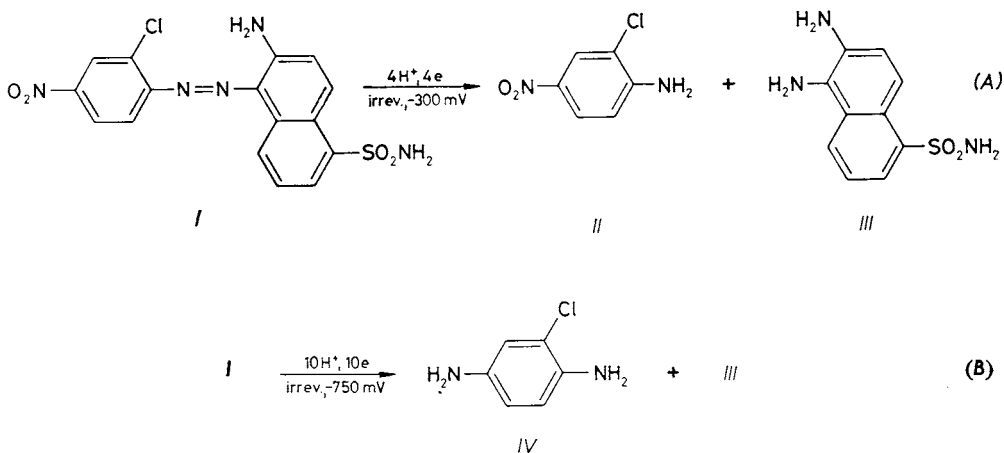
FIG. 3
TAST polarographic study of the reduction of the azo dye by coulometry at a constant i_{lim} potential of -300 mV. *a* Dependence of the limiting current of the first wave on the charge passed, Q , recalculated to the number of electrons, n , per molecule of the substance; *b* TAST polarograms of the solution after passage of charge corresponding to $n = 0$ (1), 1 (2), 2 (3), 3 (4), and 4 (5). Initial potential -100 mV



group. If 60-minute reduction in this medium at -750 mV (determined value $n = 4$) was followed by reduction at -1200 mV, then a further 4 electrons are exchanged in the next 60 minutes.

It follows from the results obtained for the TAST polarographic control of the coulometric reduction (see Fig. 3) that the first wave corresponds to the four-electron reduction. Spectrophotometric study of this reduction (see Fig. 4) indicated that it involves loss of the chromophore, i.e. the azo group.

It follows from these results that the studied azo dye exchanges a total of 10 electrons in acid medium; of these, four electrons correspond to reduction of the azo group (Eq. (A)) and the next six electrons correspond to the reduction of the nitro group, with a total exchange of 10 electrons (Eq. (B)).



In alkaline medium, the azo group is again reduced with exchange of 4 electrons to the corresponding amines (Eq. (C)), while the nitro group is reduced with ex-

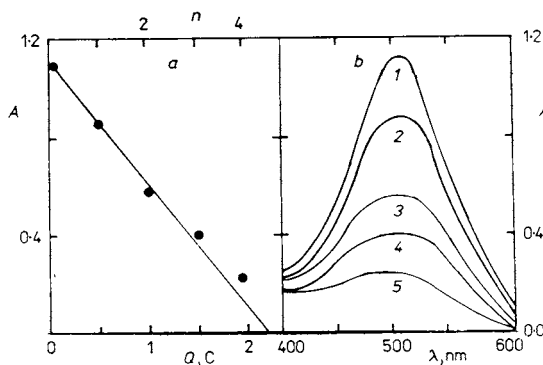
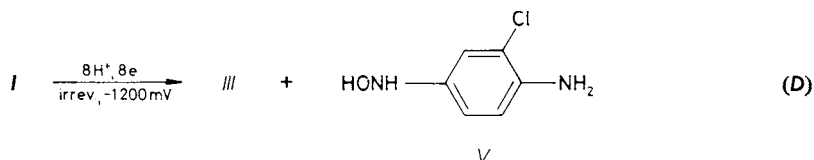
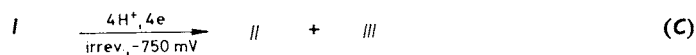


FIG. 4

Spectrophotometric study of the reduction of the azo dye by coulometry at a constant potential of -300 mV. *a* Dependence of the absorbance at 508 nm on the charge passed, Q , recalculated to the number of electrons, n , per molecule of the substance; *b* spectra of the solution after passage of current corresponding to $n = 0$ (1), 1 (2), 2 (3), 3 (4), and 4 (5).

change of 4 electrons to the hydroxylamine derivative (Eq. (D), with total consumption of 8 electrons.



Study of Adsorption Phenomena

It was demonstrated in earlier works¹⁶⁻¹⁸ that a number of azo compounds are adsorbed on the surface of a dropping mercury electrode; this phenomenon can be employed to increase the sensitivity of the determination of these substances using FSDPV or LSV at an HMDE. AC polarography and the electrocapillary curves were employed to demonstrate that the studied azo dye is also adsorbed on the surface of the DME.

In the region from 0 to -0.2 V on the AC polarogram, it was found that, in the presence of the studied azo dye, the current is decreased below the value for the base electrolyte (see Fig. 5). This fact indicates that the initial substance is adsorbed on the surface of the DME in this potential region¹⁹. It is also apparent from the measured electrocapillary curve that the azo dye is adsorbed on the surface of the DME in the region from 0 to -0.4 V (see Fig. 6). This fact was also confirmed by the increase in the peak height in FSDPV or LSV resulting from adsorptive accumulation of the azo dye on the surface of the HMDE.

The Polarographic and Voltammetric Determination of the Studied Azo Dye

Spectrophotometric study of the stability of a $1 \cdot 10^{-4} \text{ mol l}^{-1}$ solution of the azo dye in methanol revealed a decrease in the concentration by 0.7, 1.6, and 1.8% after 5, 10, and 25 days. For a $1 \cdot 10^{-5} \text{ mol l}^{-1}$ solution, this decrease was 1.8, 7.5, and 8.8% after 5, 10, and 25 days. Thus, the stock solution with a concentration of $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ was prepared fresh each week, while more dilute solutions were prepared fresh daily and were stored in the dark.

It followed from a study of the stability of variously concentrated solutions of the azo dye in the polarographed medium using DP polarography that a visible decrease in the concentration of $1 \cdot 10^{-5}$ and $1 \cdot 10^{-6} \text{ mol l}^{-1}$ solutions occurred after 30 and 20 minutes, respectively. A $1 \cdot 10^{-7} \text{ mol l}^{-1}$ solution must be measured within 10 min after preparation.

TAST and DP polarography at a DME in a Britton–Robinson buffer – methanol medium (1 : 1) with pH 2.59 yield a linear concentration dependence in the range from $5 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$ mol l⁻¹ for the first wave or peak. The height of the second peak, corresponding to the reduction of the nitro group, is a linear function of the concentration in the range from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-8}$ mol l⁻¹, while the height of the first peak can practically not be measured in this region. Table IV gives the parameters of the calibration straight lines and the calculated detection limits.

In a Britton–Robinson buffer – methanol medium (1 : 1) with pH 2.59, the following dependence of the half-wave potential on the azo dye concentration was found, apparently connected with the irreversibility of the electrode process.

<i>c</i> , mol l ⁻¹	$5 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$6 \cdot 10^{-6}$	$2 \cdot 10^{-6}$
$E_{1/2}$, mV (1st wave)	-220	-215	-205	-200
$E_{1/2}$, mV (2nd wave)	-435	-425	-415	-410

In the same medium, FSDPV yields a linear concentration dependence in the range 10^{-5} to 10^{-6} mol l⁻¹ for the 1st peak and 10^{-5} to 10^{-7} mol l⁻¹ for the second peak (see Table IV). It was further found that the peak height depends on the time elapsed between formation of the drop and the actual recording of the voltammetric curve. This fact can be explained by adsorptive accumulation of the azo dye on the surface of the HMDE. It was further found that this increase is practically independent of the accumulation potential and can be increased by stirring (see Table V).

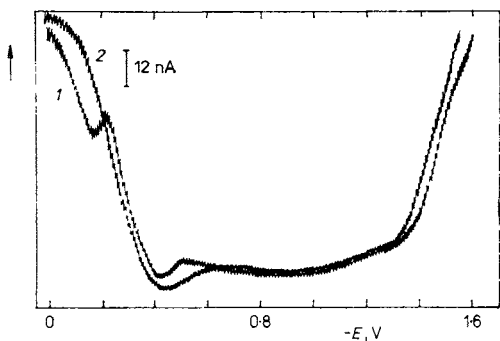


FIG. 5

AC polarogram of the azo dye ($c = 5 \cdot 10^{-5}$ mol l⁻¹) in Britton–Robinson buffer – methanol medium (1 : 1), pH 2.59 (1) and of the base electrolyte (2)

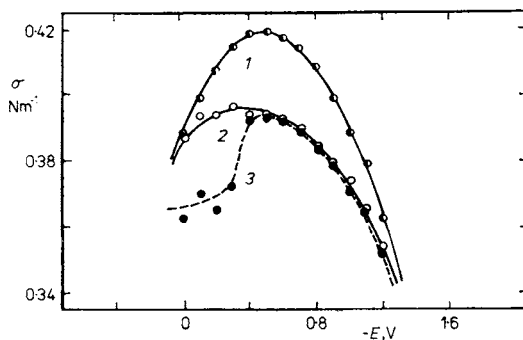


FIG. 6

Dependence of the surface tension on the potential for an aqueous solution of NaNO₃ ($c = 0.1$ mol l⁻¹) (1), for the base electrolyte (2) and for a solution of the studied substance ($c = 5 \cdot 10^{-5}$ mol l⁻¹) in the base electrolyte of Britton–Robinson buffer – methanol (1 : 1), pH 2.59 (3)

In the concentration range $(1-10) \cdot 10^{-7} \text{ mol l}^{-1}$, the calibration curve corresponding to the second peak is linear only without accumulation, i.e. when it is

TABLE IV

Parameters of the calibration straight lines and detection limits for various methods of determining the azo dye

Method	c $\mu\text{mol l}^{-1}$	Slope $\cdot 10^{-3}$ $\mu\text{A mol}^{-1} \text{ l}$	Intercept $\cdot 10^3$ μA	Correl. coef.	Detection limit $\mu\text{mol l}^{-1}$
TAST 1st wave	1-10	2.7	-1.6	0.9935	1.9
TAST 2nd wave	1-10	4.4	-2.4	0.9901	2.5
DPP 1st peak	1-10	6.1	-4.9	0.9937	1.9
DPP 2nd peak	0.1-1 ^a	8.4	-0.12	0.9896	0.2
DPP 2nd peak	0.01-0.1 ^a	13.7	0.044	0.9855	0.03
FSDPV 1st peak	1-10 ^b	13.6	-2.8	0.9998	0.4
FSDPV 1st peak	0.1-1 ^b	- ^c	-	-	-
FSDPV 1st peak	0.01-0.1 ^d	980	1.8	0.9976	0.01
FSDPV 2nd peak	0.1-1 ^b	29.8	0.2	0.9994	0.06
FSDPV 2nd peak	0.01-0.1 ^d	2 760	-1.0	0.9985	0.01
LSV 1st peak	0.01-0.1 ^d	825	5.3	0.9948	0.02
LSV 2nd peak	0.01-0.1 ^d	1 325	7.0	0.9993	0.006

^a 1st peak cannot be evaluated; ^b without accumulation (recorded immediately after drop formation); ^c peak cannot be evaluated without accumulation, dependence is nonlinear with accumulation; ^d with accumulation for 300 s with stirring (recorded 15 s after termination of stirring).

TABLE V

Dependence of the height of the 2nd peak on the accumulation time (t_a) in azo dye solution ($c = 1 \cdot 10^{-7} \text{ mol l}^{-1}$) in FSDPV on an HMDE. Medium: Britton-Robinson buffer - methanol (1 : 1), pH 2.59. Potential during accumulation, 0 V (vs SCE)

t_a s	I_p^a μA	I_p^b μA	t_a s	I_p^a μA	I_p^b μA
5	2.3	13	60	15.8	74
10	6.3	18	120	23.8	127
30	10.5	45	300	37.5	238

^a Without stirring; ^b with stirring, recorded 15 s after termination of stirring.

recorded immediately after drop formation. However, the first peak cannot be evaluated in the absence of accumulation. The second peak height is not a linear function of the concentration after accumulation for a period of 300 s with stirring. This phenomenon can be explained by the fact that the mercury surface is maximally occupied by the depolarizer and equilibrium is established between the depolarizer concentrations on the electrode surface and in solution. On the other hand, the calibration curve for the concentration range $(1-10) \cdot 10^{-8} \text{ mol l}^{-1}$ can be measured only with accumulation for 300 s with stirring. Thus, accumulation with stirring is useful for measurement of low concentrations, where reliable results cannot be obtained without accumulation; in contrast, adsorptive accumulation is not useful for higher concentrations because of the nonlinearity of the calibration curves. Table IV gives the parameters of the calibration straight lines and the calculated detection limits.

In LSV at the HMDE, the dependences of the heights of the two peaks on the pH were measured in 50% (v/v) methanol medium. The best-developed peaks were obtained at pH 2.50. At higher pH values, the peaks were sometimes larger but were less reproducible and longer accumulation times led to splitting of the 2nd peak, corresponding to the reduction of the nitro group. The dependence of the potentials of the two peaks on the pH has the same character as for the dependence of $E_{1/2}$ on the pH in TAST polarography or of E_p on the pH in DPP. The effect of the methanol content was studied using Britton-Robinson buffer with pH 1.87. It was found that the peak height increases with the methanol content in the range 10–50% (v/v) methanol. A further increase in the methanol content leads to peak splitting or deformation. The accumulation potential does not greatly affect the peak shape and height. Table VI gives the effect of stirring and of the accumulation time. It was found that the calibration curve is linear only for an accumulation time of 5 s in the concentration range $(1-10) \cdot 10^{-7} \text{ mol l}^{-1}$ for accumulation in stirred solu-

TABLE VI

Dependence of the height of the 2nd peak on the accumulation time, t_a , in an azo dye solution ($c = 2 \cdot 10^{-7} \text{ mol l}^{-1}$) in LSV at an HMDE. Medium: Britton-Robinson buffer-methanol (1 : 1), pH 2.50

t_a s	I_p^a μA	I_p^b μA	t_a s	I_p^a μA	I_p^b μA
5	8	16	60	22	125
10	10	23	120	26	158
30	15	85	300	55	241

^a Without stirring; ^b with stirring, recorded 15 s after termination of stirring.

tion. The nonlinearity of the calibration curve for longer accumulation times can be explained in terms of maximal coverage of the drop by the depolarizer, so that an increase in the concentration in solution does not lead to an increase in the adsorbed amount. Table IV lists the parameters of the calibration curves and calculated values of the detection limit in the concentration range $(1-10) \cdot 10^{-8} \text{ mol l}^{-1}$, where an accumulation time of 300 s had to be employed in a stirred solution, as the peak is too small at shorter times.

TABLE VII
Voltammetric determination of the studied azo dye after extraction

Added μg	Found, %			
	FSDPV ^a	FSDPV ^b	LSV ^a	LSV ^b
10.13	74.1	96.9	64.9	85.2
20.27	80.7	97.3	66.2	88.7
30.41	86.2	98.6	66.4	90.4
40.55	86.9	99.0	66.9	93.1

^a After three-fold extraction; ^b after six-fold extraction.

TABLE VIII
Determination of the azo dye by FSDPV and LSV at an HMDE after separation by thin-layer chromatography

Added μg	$c \cdot 10^7$ ^a mol l^{-1}	Found, %		
		FSDPV ^b	LSV ^b	LSV ^c
2.02	0.5	101.5	103.3	99.3
4.05	1	103.4	99.9	98.9
8.11	2	102.5	100.0	99.2
10.13	2.5	104.0	101.8	98.9
12.16	3	103.7	100.7	99.4
16.22	4	101.3	98.8	103.5
20.27	5	99.8	100.0	99.5
30.41	7.5	97.6	91.7	87.2
40.55	10	92.0	76.0	67.6
50.68	12.5	85.6	62.4	63.5

^a Concentration of the measured solution; ^b without accumulation, ^c with accumulation for 300 s in stirred solution.

Practical Applications

To improve the selectivity of the developed method for the determination, combination with extraction and with thin-layer chromatography were also studied.

The study of extraction as a separation or preconcentration method was begun by determining the value of the distribution ratio of the azo dye between water and diethyl ether, $D = 1.35$ (ref.²⁰). Then solutions prepared by the extraction procedure described in the experimental part from solutions of the azo dye in the range $(2-10) \cdot 10^{-7} \text{ mol l}^{-1}$ were analyzed using FSDPV and LSV at an HMDE; the concentration of the azo dye was determined from the height of the 2nd peak. The measured results were compared with the values obtained by measuring solutions prepared by addition of the appropriate volume of a $1 \cdot 10^{-4} \text{ mol l}^{-1}$ solution of the azo dye in methanol directly to 10.00 ml of the base electrolyte. It can be seen from Table VII that correct results can be obtained only by employing six-fold extraction; FSDPV should be used for the evaluation. The lower values obtained using LSV are apparently connected with the effect of traces of surface-active substances on the peak height. It would thus seem preferable to employ the standard addition method in connection with LSV.

Thin-layer chromatography carried out under the conditions given in Experimental yielded a value of $R_F = 0.25$ for the studied substance. After separation, the solutions were analyzed using FSDPV and LSV at an HMDE. The results were compared with the values obtained by measuring solutions prepared by diluting appropriate volumes of a $10^{-4} \text{ mol l}^{-1}$ solution of the azo dye in methanol with 10.00 ml of the base electrolyte. It follows from the values listed in Table VIII that very good results are obtained especially in the lower concentration range. At higher concentrations, the results are apparently affected by incomplete elution of the substance from the thin layer, so that the values found are much lower than would be theoretically expected.

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