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

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The polarographic reduction of the title azo dye was studied and optimal conditions were found for its analytical utilization in the concentration range $1 . 10^{-6}$—$1 . 10^{-7}$ mol l^{-1} using differential pulse polarography and $1 . 10^{-6}$—$1 . 10^{-8}$ mol l^{-1} using fast scan differential pulse voltammetry or linear scan voltammetry at a hanging mercury drop electrode. When the latter technique is combined with adsorptive accumulation of the studied substance on the surface of the hanging mercury drop, the determination limit can be further decreased to $3 . 10^{-9}$ mol . $. l^{-1}$.

The increasing production of azo dyes has intensified the need for analytical methods for the determination of these substances in the environment around manufacturing plants. This need has been emphasized by the possible genotoxic or ecotoxic properties of some azo compounds[1,2]. Analytical methods characterized by high sensitivity and selectivity include modern polarographic and voltammetric methods, whose utilization in the analysis of dyes and dye intermediates is discussed in the reviews[3-5]. The easy polarographic reducibility of azo compounds, whose mechanism is described in detail, for example, in the monographs[6-9], has permitted the sensitive determination of a number of azo dyes described in our earlier publications[10-13]. This work deals with the determination of 2,6-dicyano-4-nitro-2'-acetylamino-4'--diethylaminoazobenzene (see formula *I* in Eq. (*A*)), which is an industrially manufactured azo dye. So far, only the titanometric[14], spectrophotometric[15] and polarographic[16,17] determination of this substance in acetonitrile medium has been studied. Thus, the polarographic determination of this substance was studied in mixed water-methanol medium, ensuring sufficient solubility, which is easier to use than anhydrous acetonitrile. Modern techniques were used: differential pulse polarography

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(DPP) at a classical dropping mercury electrode (DME), fast scan differential pulse voltammetry (FS DPV) and linear scan voltammetry (LSV) at a hanging mercury drop electrode (HMDE) combined with adsorptive accumulation of the test substance, whose principles, capabilities and limitations are described in monographs and reviews[18-20].

EXPERIMENTAL

Reagents

The stock solution of the studied azo dye ($c = 1 . 10^{-4}$ mol l^{-1}) was prepared by dissolving a precisely weighed amount of the pure substance (Research Institute for Organic Syntheses, Pardubice-Rybitví) in p.a. methanol. More dilute solutions were prepared by precise dilution of the stock solution with methanol. All the solutions were stored in the dark. The content and purity of the substance employed were controlled titanometrically[14] and using thin-layer chromatography[15]. Britton–Robinson buffers were prepared in the usual way[21]. The actual pH value of the methanol–buffer mixture (1 : 1) was found using a combined glass and calomel GK 2320e electrode (Radiometer, Copenhagen) calibrated using acetate, borate or phosphate buffer in 50% (v/v) methanol[22,23]. The remaining chemicals were p.a. purity (Lachema, Brno). The water 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 (both from Laboratorní přístroje, Prague). A three-electrode arrangement was employed with a platinum foil auxiliary electrode and saturated calomel reference electrode, to which all the potential values are referred. Where not stated otherwise, DPP at the DME was carried out at a polarization rate of 5 mV s^{-1}, controlled drop time of 1 s and pulse height of −50 mV. A mercury reservoir height of $h = 81$ cm was employed, yielding a DME mercury flow rate of $m = 1{\cdot}34$ mg s^{-1} and drop time of $\tau = 4{\cdot}21$ s (in 0·1M-KCl at an applied potential of 0 V). FS DPV and LSV at the HMDE were carried out using an SMDE 1 static mercury drop electrode (Laboratorní přístroje, Prague) with a capillary with diameter 0·163 mm, connected as a hanging mercury drop electrode. Where not stated otherwise, FS DPV was carried out at a scan rate of 20 mV s^{-1} and modulation amplitude of −100 mV and LSV at a scan rate of 50 mV s^{-1}. Both techniques employed the maximal drop size attained by opening the valve for 160 ms.

Oxygen was removed from the analyzed solutions by bubbling with nitrogen purified by passing through an alkaline solution of sodium anthraquinone-2-sulphonate and an acidified solution of chromium(II) ions over zinc amalgam. A pre-bubbler containing a methanol–water mixture in the same ratio as in the polarographed solution 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 cathode and anode spaces separated by a frit. A mercury pool working electrode, saturated calomel reference electrode and platinum foil auxiliary electrode were used. The solution was stirred during the measurement with a magnetic stirrer and protected by an inert nitrogen atmosphere that was continuously passed over the surface of the solution.

Spectrophotometric measurements were carried out using a Pye–Unicam PU 8800 instrument (Philips) in quartz cuvettes with a thickness of 0·5 and 1 cm.

All the measurements were carried out at laboratory temperature.

Procedures

The calibration curves were measured in triplicate and evaluated by the method of linear regressions. The detection limit was calculated by the Skogerboe and Grant method[24] as the ts/a value, where s is the standard deviation of the experimental points from the calibration straight line calculated by the least squares method, a is the slope of this calibration straight line and t is the Student coefficient at the 99% confidence level.

The following procedure was employed to determine the number of electrons exchanged using constant potential coulometry: 50 ml of Britton–Robinson buffer, pH 6·0, and 48 ml of methanol were measured into the coulometric vessel (final pH 7·0) and the solution was bubbled with nitrogen with constant stirring. Simultaneously, pre-electrolysis was commenced at a selected constant potential. After about 20 min the residual current value decreased below 0·2 mA and no longer changed. Then the appropriate circuit parameters were adjusted for automatic compensation of the residual current and 2·00 ml of the solution of the studied azodye in methanol were added ($c = 1 . 10^{-4}$ mol l^{-1}), which had also been prebubbled with nitrogen. Electrolysis was terminated when the current value decreased to that of the residual current (ca after 45 to 60 min), and the charge passed was found by digital integration of the current. The reduction was studied by differential pulse polarography and spectrophotometry by removing 10 ml of solution at set intervals and measuring the differential pulse polarogram or visible spectrum.

RESULTS AND DISCUSSION

First, the stability of the stock solution of the test azo dye in methanol ($c = 1 . . 10^{-4}$ mol l^{-1}) was studied spectrophotometrically. No absorbance change ($\varepsilon = 2{\cdot}75 . 10^4$ mol^{-1} l cm^{-1}) was observed after 30 days at the wavelength of the absorption maximum ($\lambda = 606$ nm) (see Fig. 1).

Differential Pulse Polarography at the Dropping Mercury Electrode

The effect of the pH on the DP polarograms of the studied azo dye is given in Table I. Two peaks were obtained on the polarograms at all pH values except for pH 11·16 (see Fig. 2); this is connected with the presence of two electroactive groups (—NO_2 and —N=N—) in the molecule of the studied substance. A relationship was calculated for the observed shift in the peak potential (E_p) with increasing pH towards more negative values using the linear regression least squares method, E_p(mV) = $= -18{\cdot}1 - 41{\cdot}7$ pH for the first peak (more positive) and E_p(mV) = $-228{\cdot}1 - 51{\cdot}6$ pH for the second peak (more negative). A third peak appeared in the medium with the highest pH; this peak could be connected with the reduction of the nitro-group to an aminogroup. The character of the observed dependence of E_p on the pH can be explained by preliminary protonation of the azo dye, resulting in a decrease in the electron density on the electroactive functional groups, facilitating acceptance of an electron during the polarographic reduction. Analogy with the polarographic

TABLE I

The effect of the pH on the DP polarograms of the studied azo dye (*I*) ($c = 2 \cdot 10^{-6}$ mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1)

pH	1st Peak		2nd Peak	
	E_p mV	I_p nA	E_p mV	I_p nA
2·83	−130	18·7	−360	12·5
3·96	−185	15·7	−440	13·7
4·91	−215	16·2	−490	16·2
5·85	−270	19·0	−540	23·0
7·06	−320	19·7	−585	24·2
7·99	−355	17·7	−635	12·5
8·69	−375	13·0	−705	10·2
9·23	−405	7·5	−740	11·2
10·20	−445	6·7	−775	13·7
11·16[a]	−475	3·0	−785	15·7
			−850	10·0

[a] A third peak is formed.

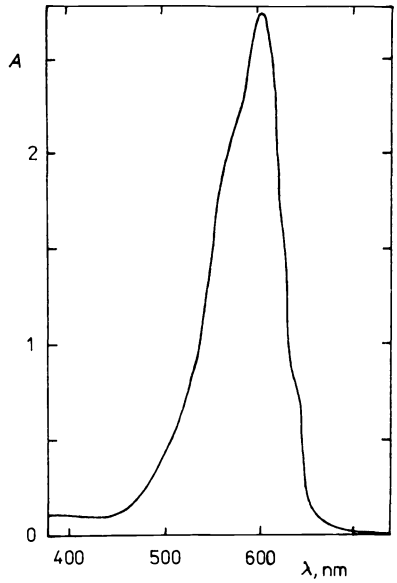


FIG. 1

Visible spectrum of the studied azo dye (*I*) in methanol ($c = 1 \cdot 10^{-4}$ mol l^{-1}) in a 1 cm cuvette

behaviour of the structurally related 2,6-dichloro-4-nitro-2'-acetylamino-4'-diethylaminoazobenzene[13] suggests that the observed changes in the height of the DPP peaks reflect changes in the reversibility of the corresponding electrode processes rather than changes in the number of electrons exchanged. The potentials of the DPP peaks of the studied azo dye with cyano groups in the 2 and 6 positions are more positive than the peak potentials of the earlier-studied azo dye[13] with chlorine atoms in the 2 and 6 positions. This difference can be explained by the fact that the –CN group for which the $-I$ and $-M$ effects are combined decrease the electron density in the region of the reduced functional groups more than the –Cl group for which the $-I$ effect is partly compensated by the $+M$ effect. The stronger electron acceptor properties of the –CN group, reflected also in the higher Hammet constant σ value for the –CN group compared with the –Cl group[25], result in easier polarographic reducibility of the azo and nitro groups in the molecule of the studied azodye (*I*) compared with the earlier-studied 2,6-dichloro analogue.

It was also confirmed that, in agreement with the theory[18], the height of the DPP peak increases with increasing modulation amplitude, electronically controlled drop time and mercury reservoir height, indicating diffusion control of the observed processes.

The irreversible character of these processes was confirmed by cyclic voltammetry at a hanging mercury drop electrode (see Fig. 3). No anodic peak was observed on the cyclic voltammograms at a polarization rate in the range $5-200$ mV s^{-1} at all the studied pH values.

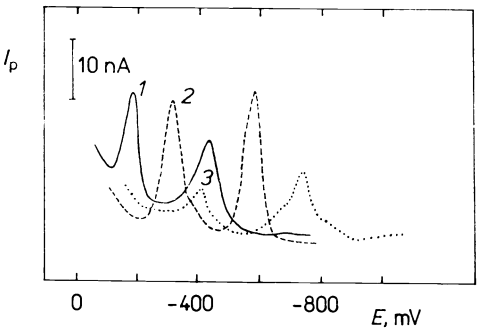


FIG. 2

Effect of the pH on the DP polarograms of a solution of the studied azo dye (*I*) ($c = 2 \cdot 10^{-6}$ mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1) of pH: *1* 3·96; *2* 7·06; *3* 9·23

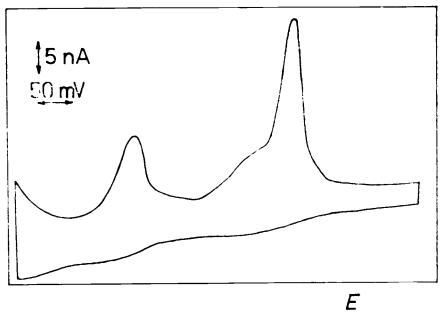


FIG. 3

Cyclic voltammogram of the studied azo dye (*I*) ($c = 2 \cdot 10^{-6}$ mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1) at pH 7·06 at a polarization rate of 50 mV s^{-1}. Initial potential -150 mV

Coulometry at a constant potential of −800 mV, corresponding to the limiting current of the second wave estimated from the DP polarogram, indicated that the charge passed in a Britton–Robinson buffer–methanol (1 : 1) medium of pH 7 for 45 min corresponds to the exchange of 8·3 electrons per molecule of the test azo dye. The charge passed at a constant potential of −500 mV for 45 min corresponds to exchange of 3·8 electrons. If the constant potential was then increased to a value of −800 mV, then the total charge passed after a further 30 min corresponded to an overall exchange of 8·1 electrons. It thus can be assumed that, under these conditions, each of the observed peaks corresponds to a four-electron reduction.

It follows from the spectrophotometric study of the reduction at constant potential of −500 mV (Fig. 4*a*) that the absorption band at 611 nm disappears, corresponding to the azo group; simultaneously, the first DPP peak disappears and the height of the second peak is practically not affected – at least at the beginning of the reduction (see Fig. 4*c*). In contrast, at a constant potential of −800 mV, the heights of both peaks decrease simultaneously (see Fig. 4*b*). The dependence of the absorbance or of the peak height on the charge passed, recalculated to the number of electrons per molecule of azo dye (*I*) intersects the abscissa at values corresponding to approximately a total exchange of 4 electrons at −500 mV and 8 electrons at −800 mV.

These observations indicate that the first peak corresponds to the 4-electron irreversible reduction of the azo group according to Eq. (*A*), while the second peak

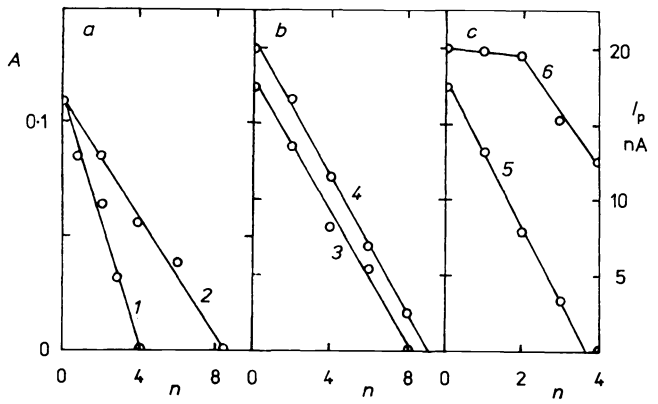
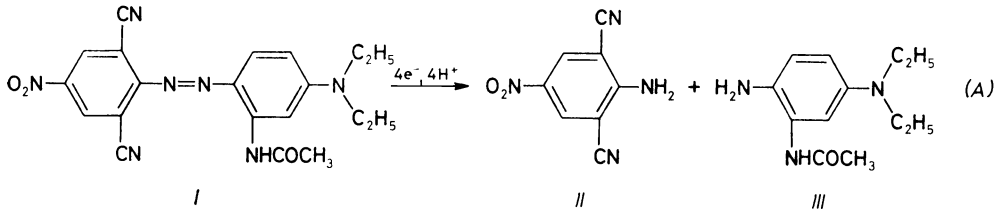


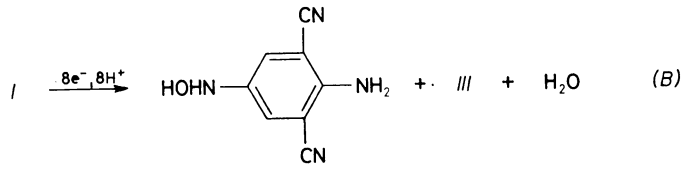
FIG. 4

Study of the coulometric reduction of the studied azo dye (*I*) by spectrophotometry (*a*) and differential pulse polarography (*b*, *c*) at pH 7·0. *a* Dependence of the absorbance at 611 nm on the charge passed recalculated to the number of electrons *n* per molecule of azo dye at a constant potential of −500 (*1*) and −800 (*2*) mV; *b* Dependence of the DP peak height at −320 mV (*3*) and −585 mV (*4*) on *n* at a constant potential of −800 mV; *c* Dependence of the DP peak height at −320 mV (*5*) and −585 mV (*6*) on *n* at a constant potential of −500 mV

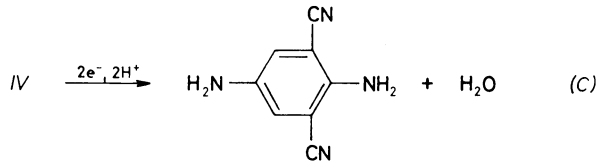
also includes the 4-electron irreversible reduction of the nitrogroup according to Eq. (*B*). The third peak that was observed only in strongly alkaline medium apparently corresponds to subsequent reduction of the temporarily formed–NHOH group to the aminogroup according to Eq. (*C*).



I *II* *III*



IV



V

This scheme is in agreement with the mechanism of the reduction of *p*-nitroazobenzene[26] and 2,6-dichloro-4-nitro-2′-acetylamino-4′-diethylaminoazobenzene[13] in which the reduction of the azo group also preceeds the reduction of the nitrogroup and also with the mechanism of the reduction of a number of aromatic aminonitrocompounds[27] in which the 4-electron reduction in acid medium is accompanied by a subsequent 2-electron reduction in alkaline medium.

From an analytical point of view, the highest and most readily evaluated peaks were obtained in medium with pH 7, where a linear dependence was obtained for the heights of both peaks on the concentration of azo dye (*I*) in the range 10^{-6} to 10^{-7} mol l^{-1}. The parameters of the calibration straight lines are given together with the calculated estimate of the detection limit in Table II. The second peak is higher but is less reproducible, so that the detection limit is about the same as that for the first peak.

A study of the stabilities of variously concentrated solutions of azo dye (*I*) in a Britton–Robinson buffer–methanol (1 : 1) solution with pH 7 indicated that the measurement should be carried out within 10 min after preparing the solution. The peak heights decrease after longer time periods (e.g. by 6% after 30 min for a $1 . 10^{-6}$ mol . $. l^{-1}$ solution).

Fast Scan Differential Pulse Voltammetry at a Hanging Mercury Drop Electrode

Table III documents the effect of the pH on the behaviour of the test azo dye (*I*) in FS DPV; once again, a pH of 7 is optimal for analytical applications. The observed shifts in the potentials of both peaks to more negative values with increasing pH can be explained analogously to DPP; however, the third peak appears at somewhat lower pH values and the height of the second peak is much greater than that of the first peak, which could be connected with "in situ" modification of the hanging mercury drop by the products of the electrode reaction. Consequently, the detection limit for the second peak is approximately half that for the first peak. The parameters

TABLE II

Parameters of the calibration curves and detection limits for the azo dye (*I*) in Britton–Robinson buffer–methanol medium (1 : 1) at pH 7·1

Method	*c* μmol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correl. coeff.	Detection limit nmol l^{-1}
DPP	0·1—1	9·0[a]	−0·8	0·9986	55
		12·4[b]	−0·5	0·9990	48
FS DPV	0·1—1	16·0	−0·6	0·9971	—
		35·5	−2·4	0·9960	—
	0·01—0·1	20·6	0·1	0·9755	24
		30·0	−0·2	0·9948	11
LSV	0·1—1	17·9	0·1	0·9951	—
		36·2	−1·7	0·9976	—
	0·01—0·1	15·5	0·0	0·9964	10
		26·2	0·1	0·9909	11
	0·001—0·01[c]	537·5	−0·2	0·9853	3
		—[d]	—	—	—

[a] The upper value always corresponds to the first peak; [b] the lower value corresponds to the second peak; [c] adsorptive accumulation for 2 min in stirred solution; recorded 15 s after termination of stirring; [d] second peak cannot be evaluated.

of the calibration straight lines and detection limits are again given in Table II, revealing a higher sensitivity for FS DPV than for DPP.

Linear Scan Voltammetry at a Hanging Mercury Drop Electrode

A medium with pH of about 7 was again found to be optimal for LSV at the HMDE. The concentration dependence is then linear from 10^{-6} to 10^{-8} mol l^{-1}, with the parameters given in Table II. The second peak is once again twice as high as the first but is less reproducible so that the detection limit is about the same as for the first peak.

The heights of both peaks depend on the time between drop formation and recording of the voltammogram, apparently as a result of adsorptive accumulation of the studied substance on the surface of the hanging mercury drop electrode. It followed from preliminary experiments that this increase is practically independent of the accumulation potential and can be enhanced by stirring the solution. Consequently, accumulation was carried out for two minutes at an applied potential of 0 V in stirred solutions, leading to a considerable decrease in the detection limit (see Table II). However, the height of the first peak must be employed in the evaluation as the height of the second peak is difficult to evaluate after adsorptive accumulation, apparently as a result of the much lower concentration of the studied substance and thus lower probability of modification of the electrode surface by the products

TABLE III

The effect of the pH on the position (E_p) and height (I_p) of the peaks of the studied azo dye (*I*) ($c = 2 . 10^{-6}$ mol l^{-1}) in FS DPV at the HMDE in Britton–Robinson buffer–methanol medium (1 : 1)

pH	1st Peak		2nd Peak		3rd Peak	
	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA
2·66	−110	16·4	−325	23·2	—	—
3·94	−170	24·8	−415	24·4	—	—
4·89	−210	23·6	−460	32·4	—	—
5·90	−250	30·0	−505	42·0	—	—
7·13	−295	32·4	−545	45·6	—	—
8·29	−335	23·0	−590	33·6	—	—
8·82	−350	22·4	−610	30·8	—	—
9·33	−370	17·6	−635	28·8	—	—
9·99	−390	8·8	−665	33·6	−775	9·2
10·80	−405	5·6	−690	32·0	−795	8·0

of the electrode reaction corresponding to the first peak. A further increase in the accumulation time was not accompanied by a significant decrease in the detection limit.

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