THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF N,N-DIMETHYL-4-AMINO-3'-NITROAZOBENZENE*

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The polarographic reduction of N,N-dimethyl-4-amino-3'-nitroazobenzene was studied by tast polarography and differential pulse polarography, constant potential coulometry and cyclic voltammetry and a mechanism was proposed for the reduction. Optimal conditions were found for the determination of the test substance using tast polarography in the concentration range 1 . 10^{-4} – 2 . 10^{-6} mol l^{-1}, differential pulse polarography at a classical dropping mercury electrode and fast scan differential pulse voltammetry at a hanging mercury drop electrode in the range 1 . 10^{-4} – 2 . 10^{-7} mol l^{-1} and linear scan voltammetry at a hanging mercury drop electrode in the range 1 . 10^{-5} – 2 . 10^{-8} mol l^{-1}. A further increase in the sensitivity was achieved through adsorptive accumulation of the test substance on the surface of the working electrode, permitting the determination to be carried out in the concentration range 1 . 10^{-7} – 2 . 10^{-9} mol l^{-1}.

The derivatives of N,N-dimethyl-4-aminoazobenzene have been demonstrated to be hepatocarcinogens[1]. The study of the metabolism or the determination of trace amounts of these substances in various matrices requires the use of exceptionally sensitive analytical methods. Because of the ready polarographic reducibility of the azo group, whose mechanism has been discussed, e.g. in monographs[2 – 4], this analysis can be carried out using modern polarographic and voltammetric methods such as differential pulse polarography (DPP) at a classical dropping mercury electrode (DME)[5], fast scan differential pulse voltammetry (FSDPV)[6] or linear scan voltammetry (LSV)[7] at a hanging mercury drop electrode (HMDE) and adsorptive stripping voltammetry (AdSV)[8], whose principles, capabilities and limitations are discussed in the cited works. These techniques have been found to be useful for the determination of N,N-dimethyl-4-aminoazobenzene[9] and its 3'-methyl[10] and 3'-halogen[11] derivatives.

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The submitted work deals with the use of these techniques for the determination of trace amounts of N,N-dimethyl-4-amino-3′-nitroazobenzene (CAS name: benzenamine, N,N-dimethyl-4[3-nitrophenyl]azo; CAS Registry No.: 3837-55-6). In addition, this genotoxic substance contains a readily reducible nitro group, whose reduction mechanism is described in detail, e.g. in reviews[12 – 15]. A mixed water–methanol medium was employed, ensuring sufficient solubility of the test substance.

EXPERIMENTAL

Reagents

The stock solution of N,N-dimethyl-4-amino-3′-nitroazobenzene (c = 1 . 10^{-3} mol l^{-1}) in methanol was prepared by dissolving 0.0270 g of the solid substance (Research Institute of Organic Syntheses, Pardubice-Rybitví) in 100 ml p.a. methanol (Lachema, Brno). More dilute solutions were prepared by dilution of this stock solution. All the solutions were stored in the dark. The purity of the substances used was controlled by TLC (ref.[16]) and HPLC (ref.[17]). It was confirmed by spectrophotometry in the visible region[18] that solutions of N,N-dimethyl-4-amino-3′-nitroazobenzene in methanol with concentrations of 1 . 10^{-3} and 1 . 10^{-4} mol l^{-1} are sufficiently stable for at least 2 months. Solutions with concentrations of 1 . 10^{-5} mol l^{-1} were prepared fresh each week and even more dilute solutions daily. The Britton–Robinson buffers were prepared in the usual manner[19]. The exact pH values of the buffers and their mixtures with methanol were controlled using a pH-meter with a glass electrode calibrated using standard aqueous buffer solutions. The other chemicals employed (chloroform, benzene, boric acid, acetic acid, phosphoric acid, perchloric acid, potassium chloride, sodium hydroxide) were of p.a. purity (Lachema, Brno). The water was doubly distilled in a quartz apparatus. The work at the lowest concentrations was carried out using methanol purified according to ref.[20]. The purified methanol was stored only in glass vessels, as contact with polyethylene led to the extraction of substances that were detrimental for the determination, especially at lower concentrations.

Apparatus

A PA 4 polarographic analyzer with a XY 4105 recorder (Laboratorní přístroje, Prague) was used in a three-electrode arrangement with a saturated silver chloride reference electrode and platinum foil auxiliary electrode. All the potential values are referred to a saturated silver chloride electrode. DC, tast and DP polarography at the DME were carried out at a potential scan rate of 5 mV s^{-1} and, where not stated otherwise, the controlled drop time was τ = 1 s, the height of the mercury reservoir was h = 36 cm and the DPP modulation amplitude was −100 mV. The dropping mercury electrode employed had the following parameters: at a mercury reservoir height of h = 36 cm, the flow rate was m = 3.35 mg s^{-1} and the drop time was τ = 2.03 s (measured in 0.1M-KCl at an applied voltage of 0 V).

FSDPV, LSV and cyclic voltammetry were carried out using an SMDE 1 mercury working electrode (Laboratorní přístroje, Prague) with a 0.140 mm diameter capillary, connected as an HMDE. Where not stated otherwise, all the measurements were carried out at a potential scan rate of 20 mV s^{-1}, maximum drop size given by opening the valve for 160 ms and FSDPV modulation amplitude of −100 mV.

Oxygen was eliminated from the test solutions by bubbling for ten minutes with nitrogen, which was purified by passage through a solution of chromium(II) ions in dilute hydrochloric acid over zinc amalgam. A prebubbler, containing the same methanol–water mixture as the test solution, was placed prior to the polarographed solution.

The acidity of the solution was measured using a PHM 62 instrument (Radiometer, Copenhagen) with glass and saturated calomel electrodes.

The spectrophotometric measurements were carried out on a PU 8800 instrument (Pye Unicam, Great Britain) in 1 cm quartz cuvettes.

The chromatographed substances were detected in TLC using a 254 nm UV lamp (Ultra-Violet Products, Inc., U.S.A.).

The coulometric measurements were carried out using a OH 404 coulometric analyzer (Radelkis, Budapest) in a 200 ml vessel with a fitted platinum wire permitting contact with the mercury pool forming the cathode. The anode was a platinum foil electrode; the cathode and anode spaces were separated by a frit. The reference electrode was always an OH 933 saturated calomel electrode (Radelkis, Budapest). The solution was stirred during the electrolysis with a magnetic stirrer and an inert atmosphere was maintained by a nitrogen stream.

The anodic oxidation was studied using an RDE 1 rotating disk electrode (Laboratorní přístroje, Prague), whose body consisted of a teflon cylinder with a diameter of 12 mm and whose electroactive part was a glassy carbon disk with a diameter of 3 mm.

All measurements were carried out at laboratory temperature.

Procedures

The calibration curves were always measured three times and evaluated by linear regression by the least squares method. The determination limit was calculated as ten times the standard deviation for 7 determinations of analyte with a concentration corresponding to the lowest point on the calibration curve[21].

The potentiostatic coulometry was carried out by transferring 40.0 ml of Britton–Robinson buffer, pH 1.89, and 50.0 ml of methanol (final pH of the water–methanol solution, 2.5) to the coulometric vessel and the solution was freed of oxygen by bubbling with nitrogen. Simultaneously, preelectrolysis was commenced at a constant potential of −800 mV corresponding to the limiting current of the second wave or −185 mV corresponding to the limiting current of the first wave of the test substance. After about 15 min, the residual current value decreased below 0.1 mA and did not further change. Then the appropriate circuit parameters were adjusted for automatic residual current compensation and 10.0 ml of the solution of N,N-dimethyl-4-amino-3′-nitroazobenzene in methanol ($c = 1 \cdot 10^{-3}$ mol l^{-1}), previously freed of oxygen, were added with constant bubbling and stirring. The electrolysis was terminated after about 60 min, when the electrolytic current once again decreased to the residual current value. The charge consumed was found by digital integration of the current passed. The reduction was followed spectrophotometrically and polarographically by taking 10 ml of solution from the coulometric vessel at the given time intervals and measuring the tast polarogram and spectrum in the visible region. The sampling was carried out prior to the beginning of the polarographic reduction and after reduction of 25, 50, 75 and 100% of the substance (calculated relative to the determined number of electrons exchanged at constant potential).

The products of the coulometric reduction were identified by TLC using the following procedure: after coulometric reduction at pH 2.5, the solution was neutralized with 0.2M-NaOH to pH 7 (controlled using universal pH paper), extracted with 2 × 10 ml chloroform and the combined extracts were condensed to 1 ml on a vacuum evaporator under decreased pressure. 30 μl of the solution formed was applied to the start of a Silufol UV 254 thin layer and developed by the ascending method using a methanol–water mobile phase (95 : 5). Detection was carried out using UV radiation and also by spraying with a 1% solution of *p*-dimethylaminobenzaldehyde in a mixture of ethanol–concentrated hydrochloric acid (95 : 5), which is a selective detection reagent for primary aromatic amines. Standards were chromatographed in parallel (3′-nitroaniline and N,N-dimethylaniline).

RESULTS AND DISCUSSION

Study of the Polarographic Behaviour of N,N-Dimethyl-4-amino-3'-nitroazobenzene

Tast polarography. First the effect of the pH on the polarographic behaviour of the test substance ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) was studied in Britton–Robinson buffer–methanol medium (4 : 6). At lower methanol concentrations, the polarographed substance was gradually precipitated from solution and the height of the observed wave decreased with time. In medium with pH < 4, the polarograms contained two different waves corresponding to the presence of two polarographically reducible groups (–N=N– and $-NO_2$) in the studied molecule. At pH > 4, the two waves coalesce to yield a single wave whose height decreases slightly with increasing pH. It can be expected that a greater number of electrons are exchanged in acid medium than in alkaline medium. The relationship $E^2_{1/2}$ (mV) = –116 – 48 pH (correlation coefficient 0.9907) was found for the more negative wave at pH 2 – 12. This shift of $E_{1/2}$ with decreasing pH to more positive values can be explained on the basis of preliminary protonation of the substance, leading to a decrease in the electron density in the region of expected electron transfer with facilitation of the reduction. The determined values of the slope of the logarithmic analysis of the curves obtained (see Table I) indicate that both the waves correspond to irreversible processes. This fact was also confirmed by the

TABLE I

The effect of the pH on the tast polarograms of N,N-dimethyl-4-amino-3'-nitroazobenzene ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) in mixed Britton–Robinson buffer–methanol (4 : 6) medium

pH[a]	$E^1_{1/2}$ mV	I^1_{lim} μA	α_1[b] mV	$E^2_{1/2}$ mV	I^2_{lim} μA	α_2[b] mV
2.8	-55	0.77	38.3	-255	3.42[c]	56.3
3.6	-110	0.73	46.1	-320	3.50[c]	64.5
4.9	–[d]	–[d]	–[e]	–[e]	3.60	–[e]
6.0	–[d]	–[d]	–[e]	-400	3.50	91.0
7.4	–[d]	–[d]	–[e]	-470	2.55	80.9
8.6	–[d]	–[d]	–[e]	-500	2.50	76.1
8.9	–[d]	–[d]	–[e]	-540	2.35	80.6
9.5	–[d]	–[d]	–[e]	-550	2.30	76.7
10.2	–[d]	–[d]	–[e]	-610	2.32	72.3
10.9	–[d]	–[d]	–[e]	-650	2.34	67.0
12.1	–[d]	–[d]	–[e]	-680	2.38	57.8

[a] pH of the Britton–Robinson buffer–methanol (4 : 6) mixture. [b] Slope of the logarithmic analysis. [c] Limiting current corresponding to the sum of the first and second waves. [d] The two polarographic waves coalesce. [e] The logarithmic analysis cannot be carried out because of the poor resolution of the two waves.

determined dependence of the half-wave potential on the concentration of the test substance (see Table II). It is apparent from the comparison of the slope of the logarithmic analysis and the slope of the $E_{1/2}$ dependence that this is not a simple irreversible process with rapid preliminary protonation, but rather a more complicated process, which certainly includes a role of adsorption of the test substance on the surface of the working electrode. At pH < 4 a maximum appears in the region of the second wave, and can be suppressed by the addition of 50 μl of 0.5% Triton X-100 solution in methanol to 10 ml of the polarographed solution. The dependence of the height of the wave on the square root of the height of the mercury reservoir in Britton–Robinson buffer–methanol (4 : 6) medium is linear for both the first and the second wave, but does not pass through the origin for the first wave, so that the current is at least partly kinetically controlled. However, it may be that the waves are poorly resolved, leading to difficulties in measuring their heights. The dependences of the heights of both waves on the concentration of N,N-dimethyl-4-amino-3′-nitroazobenzene is linear in the given medium from 1 . 10^{-4} to 2 . 10^{-6} mol l^{-1}.

Constant potential coulometry. It was found by coulometry at a constant potential of −800 mV that the current passed in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.6 after 60 min corresponds to the exchange of 8.33 F mol^{-1}. At constant potential of −195 mV in this medium, the current passed after 60 min corresponded to exchange of 4.44 F mol^{-1}.

At a constant potential of −800 mV in Britton–Robinson buffer–methanol (4 : 6) medium at pH 11.0, the charge passed after 90 min corresponds to exchange of 5.76 F mol^{-1}.

It follows from the spectrophotometric study of the reduction in medium with pH 2.5 at a constant potential of −195 mV (Fig. 1*a*) that the absorption of the azo group decreases sharply at 460 nm. In contrast, the absorption at about 250 nm slightly increases. The gradual changes in the spectra with time after completion of the electrolysis accompanied by the presence of an isosbestic point indicate that the substance formed in the reduction can undergo subsequent chemical reactions. The dependence of the absorbance at 460 nm on the charge passed is linear and intercepts the abscissa at the

TABLE II

Dependence of the half-wave potential of N,N-dimethyl-4-amino-3′-nitroazobenzene on its concentration in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.8

Potential	*c*, mol l^{-1}			
	1 . 10^{-4}	4 . 10^{-5}	1 . 10^{-5}	4 . 10^{-6}
$E^1_{1/2}$, mV	−55	−50	−45	−40
$E^2_{1/2}$, mV	−255	−235	−215	−200

value 4.4 F mol^{-1}. At a constant potential of –800 mV, the absorption band at 460 nm, corresponding to the azo group, completely disappears. The absorption band at about 250 nm, which apparently corresponds to the amines formed, increases slightly, indicating a more complex reaction mechanism.

It follows from the tast polarographic study of the coulometric reduction at a constant potential of –195 mV (see Fig. 1*b*) that the first wave disappears, accompanied by a decrease in the height of the second wave, apparently as a consequence of partial reduction of the nitro group at the constant potential employed. The dependence of the limiting current of the first wave on the charge passed is linear and intercepts the horizontal axis at a value of 4.4 F mol^{-1}.

At a constant potential of –800 mV, the first wave disappears more rapidly than the second wave. The dependence of the heights of both waves on the charge passed are once again linear and intercept the horizontal axis at a value of 4.2 F mol^{-1} for the first wave and 8.4 F mol^{-1} for the second wave. No anodic wave appeared in the tast polarographic study of the coulometric reduction.

An attempt was also made to identify the products of the coulometric reduction in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.5 using thin-layer chromatography and the procedure described under Experimental. The chromatogram of the solution obtained from the reduction at –195 mV visually contained a yellow spot (R_F = 0.70), which was also visible under UV light. This spot turned orange after spraying with a solution of *p*-dimethylaminobenzaldehyde. It was demonstrated by comparison with the standard substance that this substance is 3′-nitroaniline. A further spot with R_F = 0.54 apparently corresponds to N,N-dimethyl-*p*-phenylendiamine (identical behaviour of the standard substance in thin-layer chromatography and detection). However,

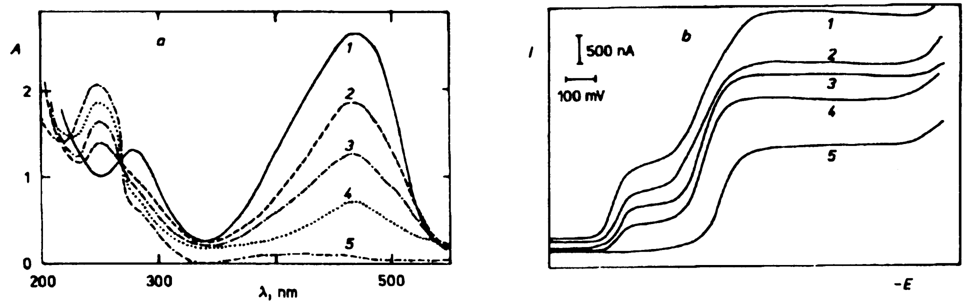


FIG. 1

Spectra (*a*) and tast polarograms (*b*) of solutions of N,N-dimethyl-4-amino-3′-nitroazobenzene (c = 1 . 10^{-4} mol l^{-1}) measured during the reduction by constant potential coulometry at –195 mV in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.5 for passage of charge (F mol^{-1}): *1* 0; *2* 1.1; *3* 2.3; *4* 3.4; *5* 4.4. Cuvette thickness 1 cm this detection lies at the detection limit as a consequence of the low intensity of the quenching of the fluorescence of the background and the colour formed after spraying with the *p*-dimethylaminobenzaldehyde solution.

The proof of the presence of N,N-dimethyl-*p*-phenylendiamine in the solution after reduction at −800 mV was also close to the detection limit, while 3′-nitroaniline was not detected in the solution. However, the chromatogram obtained was shown to contain a further tailing spot with $R_F = 0.41$, detectable under UV light, which turned yellow after spraying with a *p*-dimethylaminobenzaldehyde solution, indicating the presence of a primary amino group on the aromatic ring.

In addition, the identity of the products of the coulometric reduction under the above conditions was confirmed by anodic voltammetry at a rotating glassy carbon disk electrode. The polarization curves of N,N-dimethyl-4-amino-3′-nitroazobenzene ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) were measured at a polarization rate of 5 mV s^{-1} and electrode rotation rate of 2 000 r.p.m. in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.7, yielding a single anodic wave with a half-wave potential of 0.91 V. Under the same conditions, 3′-nitroaniline yielded a single anodic wave with a half-wave potential of 1.24 V and N,N-dimethyl-*p*-phenylendiamine yielded two anodic waves with half-wave potentials of 0.34 V and 0.60 V. These curves were compared with the curves of a solution of N,N-dimethyl-4-amino-3′-nitroazobenzene after coulometric reduction at constant potential of −195 mV, and three waves were found ($E^1_{1/2} = 0.33$ V, $I^1_{\mathrm{lim}} = 5$ μA; $E^2_{1/2} = 0.59$ V, $I^2_{\mathrm{lim}} = 26$ μA; $E^3_{1/2} = 1.24$ V, $I^3_{\mathrm{lim}} = 47$ μA). The first two waves can be assigned to the oxidation of N,N-dimethyl-*p*-phenylendiamine, while the third wave apparently corresponds to the oxidation of 3′-nitroaniline. Three waves were observed in the solution of N,N-dimethyl-4-amino-3′-nitroazobenzene after reduction at constant potential of −800 mV, of which the first two ($E^1_{1/2} = 0.31$ V and $E^2_{1/2} = 0.59$ V) corresponded to N,N-dimethyl-*p*-phenylendiamine, while the third poorly developed wave in the region around 1.2 V corresponds to the second product of the coulometric reduction.

Cyclic voltammetry at a hanging mercury drop electrode. The cyclic voltammograms of N,N-dimethyl-4-amino-3′-nitroazobenzene ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) and 3-nitroaniline ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) as the expected product of the electrochemical reduction were measured in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.7, 7.0 and 12.2 at polarization rates of 2 – 50 mV s^{-1}. It is apparent from Fig. 2*a* that a multistep irreversible process occurs under these conditions at pH 2.7, where the peaks at −50 and −100 mV apparently correspond to the reduction of the azo group, while that at −270 mV corresponds to the reduction of the nitro group. The narrow, sharp peak at −50 mV is apparently connected with the adsorption of the test substance on the surface of the working electrode. It can be seen from Fig. 2*b* that, at pH 7.0, the reduction of the azo and nitro groups coalesce into a single process, where the sharp, narrow peak at a potential of about −435 mV is apparently connected with the adsorption of the test substance. It is further apparent from Fig. 2*b* that 3′-nitroaniline itself is reduced at a more negative potential. It can be expected that the first step in the reduction of N,N-dimethyl-4-amino-3′-nitroazobenzene does not involve the splitting of the azo group to form 3′-nitroaniline. The process remains irreversible. The anodic peak at –0.8 V is apparently connected with the reverse oxidation of the reduction product of the nitro group, as can be observed for both N,N-dimethyl-4-amino-3′-nitroazobenzene and 3′-nitroaniline. It follows from Fig. 2*c* that the process becomes quasi-reversible in alkaline medium. The considerable potential difference between the more negative cathodic peak of N,N-dimethyl-4-amino-3′-nitroazobenzene and the cathodic peak of 3′-nitroaniline again indicates that the first step in the reduction does not involve the splitting of the azo group with the formation of 3′-nitroaniline. The sharp, narrow, more positive peak of N,N-dimethyl-4-amino-3′-nitroazobenzene is again assigned to adsorption of this substance on the surface of the working electrode. The anodic peak at –0.34 V is

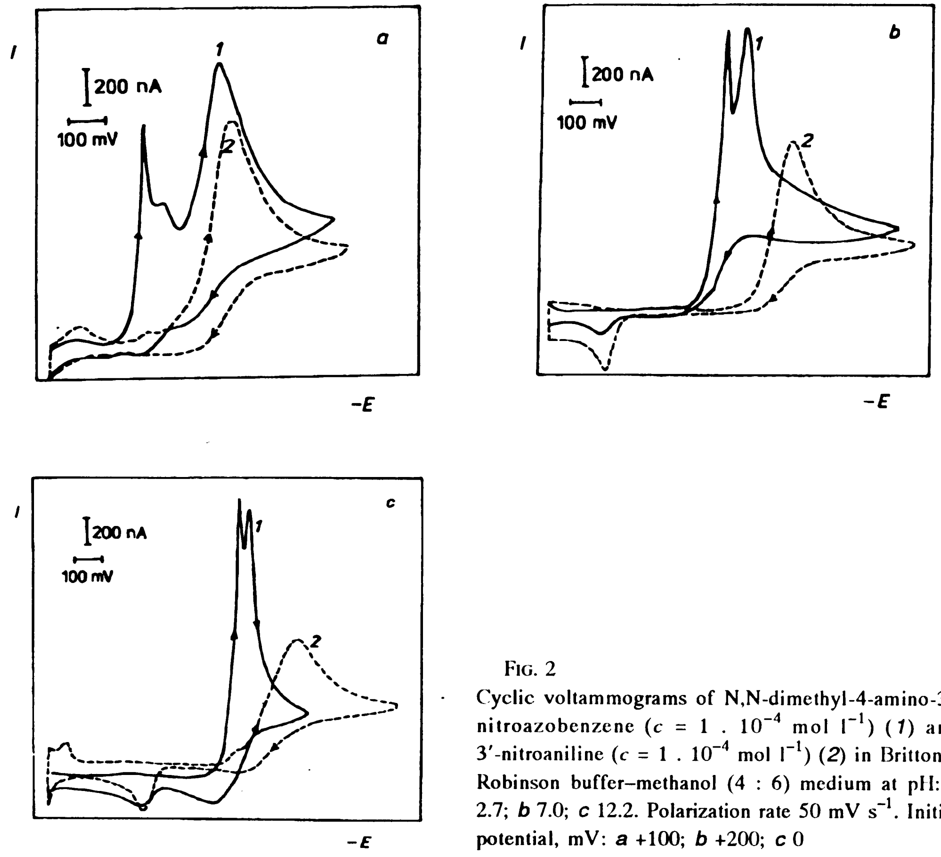
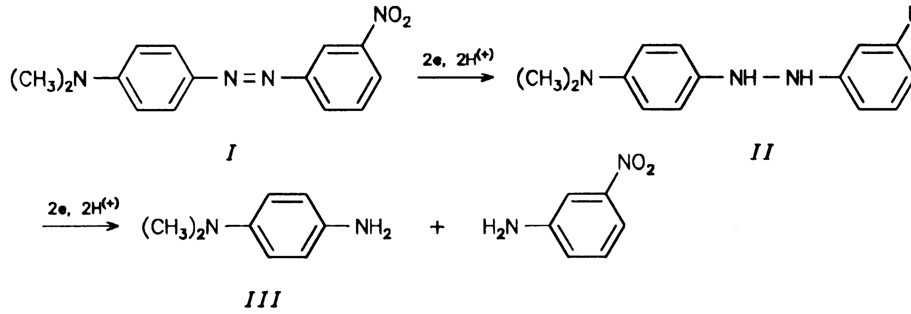


FIG. 2

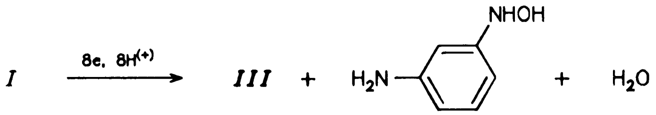
Cyclic voltammograms of N,N-dimethyl-4-amino-3′-nitroazobenzene (c = 1 . 10^{-4} mol l^{-1}) (*1*) and 3′-nitroaniline (c = 1 . 10^{-4} mol l^{-1}) (*2*) in Britton–Robinson buffer–methanol (4 : 6) medium at pH: *a* 2.7; *b* 7.0; *c* 12.2. Polarization rate 50 mV s^{-1}. Initial potential, mV: *a* +100; *b* +200; *c* 0 apparently connected with reoxidation of the production of reduction of the nitro group, as it is also present for 3′-nitroaniline alone. The anodic peak at –0.6 V is apparently connected with the quasireversible reduction of the azo group to a hydrazo group.

Proposed reduction mechanism. It can be assumed on the basis of the above information that four-electron reduction of N,N-dimethyl-4-amino-3′-nitroazobenzene occurs in acid medium at the potential of the limiting current of the first wave according to Eq. (*A*), while eight-electron reduction occurs at the limiting current of the second wave according to Eq. (*B*).



I *II* *III*

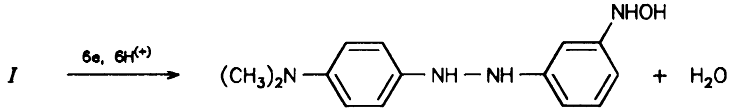
(*A*)



(*B*)

As the pH increases, the waves coalesce and the rate of reduction of the temporarily formed hydrazo compound (*II*) decreases, leading to a gradual decrease in the overall height of the consequent wave to a value corresponding to the exchange of six electrons.

In alkaline medium, the azo group is reduced only to the hydrazo group (similarly as in the previously described reduction of 4-nitroazobenzene[22,23]), where the corresponding wave coalesces with the wave of the four-electron reduction of the nitrogroup.



(*C*)

The proposed mechanism is also supported by the fact that the position and height of the wave of N,N-dimethyl-4-aminoazobenzene in medium with pH 2.7 roughly corresponds to the position and height of the first wave of N,N-dimethyl-4-amino-3′-nitroazobenzene measured under the same conditions.

Analytical Utilization of the Polarographic Reduction of N,N-Dimethyl-4-amino-3′-nitroazobenzene

Tast polarography. For analytical purposes, it is optimal to work at pH 2.7, where the determination can be based on both the height of the first wave as well as on the sum of the heights of the first and second waves, which exhibit a linear dependence on the concentration of N,N-dimethyl-4-amino-3′-nitroazobenzene in the range $1 \,.\, 10^{-4}$ – $2 \,.\, 10^{-6}$ mol l^{-1}. The parameters of these dependences are collected in Table III.

Differential pulse polarography. In the acidic region, the test substance yields two peaks that gradually coalesce with increasing pH (see Fig. 3). It can be seen that the dependence of the peak potential E_p on the pH retains the same character as the dependence of $E_{1/2}$ on the pH·in tast polarography. The method of linear regression was employed to calculate the parameters E_p^1 (mV) = −128 – 70 pH (correlation coefficient 0.9990) for the first peak and E_p^2 (mV) = −148 – 42 pH (correlation coefficient 0.9932) for the second peak. However, the dependence of the peak height on the pH is more a result of the effect of the pH on the steepness of the polarographic wave than on its height. (The peak height was then measured from the line connecting the minima prior to the first peak and after the second peak). Under these conditions, the dependence of the peak height on the concentration of N,N-dimethyl-4-amino-3′-nitroazobenzene is linear in the range $1 \,.\, 10^{-4}$ – $2 \,.\, 10^{-6}$ mol l^{-1}. At lower concentrations, the presence of the peaks of trace impurities on the curve of the base electrolyte alone made it necessa-

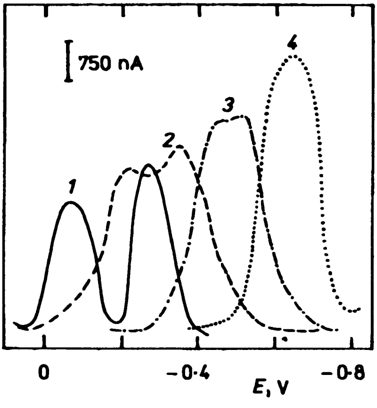


FIG. 3
Differential pulse polarograms of N,N-dimethyl-4-amino-3′-nitroazobenzene (c = 1 . 10^{-4} mol l^{-1}) in Britton–Robinson buffer–methanol (4 : 6) medium at pH: *1* 2.7; *2* 4.9; *3* 8.5; *4* 12.1

ry to employ prepurified Britton–Robinson buffer–methanol (1 : 9) medium, with a final pH of 2.1. However, then only the second peak could be used for the determination, as the first peak coincides with the dissolution of mercury. The parameters of the calibration straight lines obtained are again collected in Table III.

Fast scan differential pulse voltammetry. The effect of the pH on the FSDP voltammograms of N,N-dimethyl-4-amino-3′-nitroazobenzene was studied in Britton–Robinson buffer–methanol (4 : 6) medium at a depolarizer concentration of 1 . 10^{-5} mol l^{-1}. In acid medium, the test substance yields two peaks, while only one peak is obtained in neutral or alkaline medium. The second peak, whose height decreases with increasing pH, is much lower than the first peak (see Table IV), apparently as a consequence of passivation of the working electrode, whose surface is not renewed during the measurement. It holds for the first peak in the range pH 2 – 12 that E_p (mV) = 137 – 73.5 pH. Britton–Robinson buffer–methanol (4 : 6) medium at pH 8.4 is optimal for analytical applications as it yields peaks with favourable shape, height and ease of evaluation that are sufficiently separated from the impurity peaks on the background of the base electrolyte. The test substance yields a single peak in this medium, whose height is a

TABLE III
Parameters of the calibration curves and determination limits for N,N-dimethyl-4-amino-3′-nitroazobenzene in Britton–Robinson buffer–methanol (4 : 6) medium at pH 2.7

Method	c mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r[a]	L_Q[b] mol l^{-1}
Tast[c]	(1 – 10) . 10^{-5}	7.0	2.0	0.9942	–
	(2 – 10) . 10^{-6}	7.1	–2.7	0.9978	1.1 . 10^{-6}
Tast[d]	(1 – 10) . 10^{-5}	34.2	–3.0	0.9997	–
	(2 – 10) . 10^{-6}	31.8	–0.5	0.9991	0.7 . 10^{-6}
DPP[e]	(1 – 10) . 10^{-5}	21.6	28.0	0.9952	–
	(2 – 10) . 10^{-6}	19.0	–10.0	0.9954	1.6 . 10^{-6}
DPP[f]	(1 – 10) . 10^{-5}	32.4	11.0	0.9998	–
	(2 – 10) . 10^{-6}	39.5	–1.0	0.9991	0.7 . 10^{-6}
DPP[f,g]	(2 – 10) . 10^{-7}	66.0	12.0	0.9959	1.5 . 10^{-7}

[a] Correlation coefficient. [b] Determination limit. [c] Parameters corresponding to the height of the first wave. [d] Parameters corresponding to the sum of the heights of the first and second waves. [e] Parameters corresponding to the height of the first peak. [f] Parameters corresponding to the height of the second peak. [g] Britton–Robinson buffer–methanol (9 : 1) medium at pH 2.1.

linear function of the concentration of N,N-dimethyl-4-amino-3′-nitroazobenzene in the range 1 . 10^{-4} – 2 . 10^{-7} mol l^{-1} (see Table V).

TABLE IV
The effect of the pH on the FSDP voltammograms of N,N-dimethyl-4-amino-3′-nitroazobenzene (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer–methanol (4 : 6) medium

pH[a]	E_p^1, mV	I_p^1, μV	E_p^2, mV	I_p^2, μV
2.6	-65	0.44	-260	0.16
3.5	-115	0.51	-290	0.16
4.9	-200	0.47	-370	0.13
5.9	-275	0.71	-425	0.07
7.3	-450	0.92	–[b]	–[b]
8.4	-495	0.78	–[b]	–[b]
8.9	-520	0.79	–[b]	–[b]
9.2	-540	0.66	–[b]	–[b]
9.9	-590	0.85	–[b]	–[b]
10.7	-650	0.91	–[b]	–[b]
12.2	-665	0.80	–[b]	–[b]

[a] pH of the Britton–Robinson buffer–methanol (4 : 6) medium. [b] The two peaks coalesce.

TABLE V
Parameters of the calibration straight lines and determination limits for N,N-dimethyl-4-amino-3′-nitroazobenzene obtained by FSDPV at the HMDE in Britton–Robinson buffer–methanol (4 : 6) medium at pH 8.4

c mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r[a]	L_Q[b] mol l^{-1}
(1 – 10) . 10^{-5}	8.10	0.2	0.9984	–
(1 – 10) . 10^{-6}	8.40	-0.8	0.9999	–
(2 – 10) . 10^{-7}	8.60	0.4	0.9982	1.0 . 10^{-7}
(2 – 10) . 10^{-8} [c]	135	0.1	0.9995	0.9 . 10^{-8}
(2 – 10) . 10^{-9} [d]	585	-0.3	0.9997	2.2 . 10^{-9}

[a] Correlation coefficient. [b] Determination limit. [c] Accumulation potential –300 mV, accumulation time 5 min in unstirred solution. [d] Accumulation potential –300 mV, accumulation time 5 min in stirred solution.

The following dependences of the height of the FSDPV peak I_p on the time t between formation of the hanging mercury drop and the recording of the FSDP voltammogram were found in the above medium in unstirred solution at a working electrode potential of –300 mV at a N,N-dimethyl-4-amino-3′-nitroazobenzene concentration of 1 . 10^{-7} mol l^{-1}:

t, s	10	60	180	300	600	900
I_p, nA	1.3	3.0	8.3	13.3	20.9	29.0

This dependence can be explained on the basis of adsorptive accumulation of the test substance on the surface of the working electrode, which can be utilized to greatly increase the sensitivity of the determination. Adsorptive accumulation for 5 min in unstirred solution yields a linear concentration dependence in the range (2 – 10) . 10^{-8} mol l^{-1} in unstirred solution, and in the range (2 – 10) . 10^{-9} mol l^{-1} in stirred solution (see Table V).

Linear Scan Voltammetry at a Hanging Mercury Drop Electrode

The effect of the pH on the behaviour of the test substance in LSV at the HMDE is given in Table VI. It can be seen that the highest peaks were obtained in medium at pH

TABLE VI
The effect of the pH on the LS voltammograms of N,N-dimethyl-4-amino-3′-nitroazobenzene (c = 1 . 10^{-6} mol l^{-1}) in Britton–Robinson buffer–methanol (4 : 6) medium

pH[a]	E_p^1, mV	I_p^1, μA	E_p^2, mV	I_p^2, μA
2.6	-110	0.090	-310	0.047
3.5	-175	0.107	-340	0.050
4.9	-285	0.163	–[b]	–[b]
5.8	-350	0.247	–[b]	–[b]
7.3	-440	0.185	–[b]	–[b]
8.4	-475	0.165	–[b]	–[b]
8.8	-500	0.155	–[b]	–[b]
9.2	-535	0.145	–[b]	–[b]
10.7	-630	0.185	–[b]	–[b]
12.3	-700	0.314	–[b]	–[b]

[a] pH of the Britton–Robinson buffer–methanol (4 : 6) medium. [b] The two peaks coalesce.

5.8 and 12.3. The dependence of the peak height on the depolarizer concentration under these conditions is linear in the range 1 . 10^{-5} – 2 . 10^{-8} mol l^{-1} (see Table VII). However, at concentrations of (2 – 10) . 10^{-8} mol l^{-1} it is preferable to employ five-minute adsorption accumulation in unstirred solution. Adsorption accumulation for the same period in stirred solution permits the determination to be carried out in the concentration range (2 – 10) . 10^{-9} mol l^{-1} (see Table VII).

TABLE VII
Parameters of the calibration straight lines and determination limits for N,N-dimethyl-4-amino-3′-nitroazobenzene obtained by LSV at the HMDE in Britton–Robinson buffer–methanol (4 : 6) medium

pH[a]	c mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r[b]	L_Q[c] mol l^{-1}
5.8	(1 – 10) . 10^{-6}	24.7	27.7	0.9966	–
	(1 – 10) . 10^{-7}	23.2	0.8	0.9926	–
	(2 – 10) . 10^{-8}	20.2	-0.05	0.9946	1.7 . 10^{-8}
5.8[d]	(2 – 10) . 10^{-9}	257	0.2	0.9963	1.4 . 10^{-9}
12.3	(1 – 10) . 10^{-6}	32.3	2.6	0.9991	–
	(1 – 10) . 10^{-7}	31.0	0.2	0.9973	–
	(2 – 10) . 10^{-8}	27.0	0.2	0.9977	1.1 . 10^{-8}
12.3[e]	(2 – 10) . 10^{-9}	455	0.3	0.9959	1.5 . 10^{-9}

[a] pH of the Britton–Robinson buffer–methanol (4 : 6) mixture; [b] Correlation coefficient. [c] Determination limit. [d] Accumulation potential -300 mV, accumulation time 300 s in unstirred solution. [e] Accumulation potential -300 mV, accumulation time 300 s in stirred solution.

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