

## THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF THE 4'-HALODERIVATIVES OF N,N-DIMETHYL-4-AMINO-AZOBENZENE\*

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The polarographic reduction of 4-haloderivatives of N,N-dimethyl-4-aminoazobenzene has been studied in mixed water-methanol medium, a mechanism has been proposed and optimal conditions have been found for determination of these substances by TAST polarography with a determination limit of about  $1 \cdot 10^{-6} \text{ mol l}^{-1}$ , by differential pulse polarography with a determination limit of about  $1 \cdot 10^{-7} \text{ mol l}^{-1}$  and linear scan voltammetry with a determination limit of about  $5 \cdot 10^{-8} \text{ mol l}^{-1}$ . In the latter technique, the determined substance was accumulated by adsorption on the surface of a hanging mercury drop electrode to decrease the determination limit below  $1 \cdot 10^{-8} \text{ mol l}^{-1}$ .

The 4'-haloderivatives of N,N-dimethyl-4-aminoazobenzene have been suspected of chemical carcinogenicity<sup>1,2</sup>. Even very small amounts of these substances can have a detrimental effect on biological processes and thus a great deal of attention has recently been paid to analytical methods useful for determination of trace amounts of these substances in both biological samples and in working or the natural environment<sup>3</sup>. The methods successfully used for this purpose in the last decade include modern polarographic and voltammetric methods<sup>4</sup>, which have been used to determine various genotoxic derivatives of azobenzene<sup>4-9</sup> and other biologically important azocompounds<sup>10,11</sup>.

This work describes the determination of the 4-haloderivatives of N,N-dimethyl-4-aminoazobenzene (X = F, Cl, Br or I) by TAST and differential pulse (DPP) polarography using a classical mercury drop electrode (DME) and using linear scan voltammetry (LSV) at a hanging mercury drop electrode (HMDE). It follows from the theory of these methods<sup>12</sup> that they are more sensitive than the classical methods. Practical applications of these new methods should be based on knowledge of the behaviour of these substances in classical polarography, given in the monographs<sup>13-15</sup> for azocompounds.

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## EXPERIMENTAL

### Reagents

The stock solution of the studied 4-haloderivatives of N,N-dimethyl-4-aminoazobenzene in methanol ( $c = 1 \text{ mmol l}^{-1}$ ) was prepared by dissolving a precisely weighed amount of the solid substance (Research Institute for Organic Synthesis, Pardubice-Rybitví) in p.a. methanol. Solutions with lower concentrations were prepared by precise dilution of the stock solution. All solutions were stored in the dark. The purity of the prepared substances was controlled using TLC<sup>16</sup> and HPLC<sup>17</sup>. The concentrations of the stock solutions were controlled titanometrically<sup>18</sup>.

The Triton X-100 (alkylphenylpolyethyleneglycol, Serva, Heidelberg) was prepared by dissolving 0.5 ml of the substance in 100 ml methanol. The Britton-Robinson buffer solutions were prepared in the usual manner<sup>19</sup>. The chemicals employed (methanol, phosphoric acid, acetic acid, boric acid, sodium hydroxide) were of p.a. purity (Lachema, Brno). The water employed was doubly distilled in a quartz apparatus.

### Apparatus

Measurements were carried out using a PA 2 polarographic analyzer with an XY 4 105 recorder (both from Laboratorní přístroje, Prague). DC, TAST and DP polarography were carried out using a classical dropping mercury electrode with the following parameters: at a mercury reservoir height of  $h = 36 \text{ cm}$ , the flow rate was  $m = 1.61 \text{ mg s}^{-1}$  and drop time  $t = 2.09 \text{ s}$  (measured in 0.1M-KCl with an applied voltage of 0V vs SCE). Where not stated otherwise, a polarization rate of  $5 \text{ mV s}^{-1}$ , mercury reservoir height of 36 cm, electronically controlled drop time of 1 s and DPP pulse modulation amplitude of  $-100 \text{ mV}$  were employed. Linear scan voltammetry was carried out using a static mercury drop electrode SMDE 1 (Laboratorní přístroje, Prague) with a capillary with a diameter of 0.136 mm, connected as a hanging mercury drop electrode (HMDE). Where not stated otherwise, a polarization rate of  $20 \text{ mV s}^{-1}$  and maximum drop size attained by opening the mercury valve for 160 ms were employed. All measurements were carried out using a three-electrode system with a saturated calomel reference electrode and platinum foil auxiliary electrode with an area of about  $1 \text{ cm}^2$ . All the potential values are related to the SCE. Oxygen was removed from the analyzed solution by bubbling for ten minutes with nitrogen purified by passing through an alkaline solution of sodium anthraquinone-2-sulphonate and an acidic chromium(II) ion solution over zinc amalgam. A prebubbling methanol-water (9 : 1) mixture was placed prior to the polarographic vessel.

Coulometric measurements were carried out using an OH 404 coulometric analyzer (Radelkis, Budapest) with automatic residual current compensation and digital integration of the charge passed. An all-glass vessel with a volume of 200 ml was employed, with a mercury pool cathode, platinum foil with an area of  $5 \text{ cm}^2$  as the anode and saturated calomel reference electrode. The cathode and anode spaces were separated by a frit, the solution was stirred by a magnetic stirrer during the measurements and nitrogen was passed over the surface of the solution to ensure an inert atmosphere.

Spectrophotometric measurements were carried out using a PU 8 800 instrument (Pye Unicam, England) in quartz cuvettes with a thickness of 0.5 cm.

The solution acidity was measured using a PHM 62 instrument (Radiometer, Copenhagen) with a glass indicating and saturated calomel reference electrode. The pH measurements of the methanol-buffer solutions (9 : 1) were calibrated using oxalate and succinate buffers in 90 vol. % methanol<sup>20</sup>.

All measurements were carried out at laboratory temperature.

### Procedure

The polarographed solutions were prepared by measuring the appropriate volume of a solution of the studied substance in methanol into a volumetric flask with a volume of 10 ml; 8.0 ml of methanol and 1.0 ml of the appropriate Britton–Robinson buffer were added and the solution was diluted to the mark with methanol. Calibration curves were measured three times and evaluated by the linear regression method. The determination limit was found by the Skogerboe and Grant method<sup>21</sup> as the  $ts/a$  value, where  $s$  is the standard deviation of the experimental points from the calibration straight line calculated by the method of linear regression,  $a$  is the slope of this straight line and  $t$  is the Student coefficient for 99% reliability, depending on the number of points employed to construct the calibration curve.

The reduction was studied using potentiostatic coulometry by measuring 80.0 ml methanol, 10.0 ml Britton–Robinson buffer with the required pH into the coulometric vessel and bubbling with nitrogen. Pre-electrolysis at constant potential was simultaneously commenced. After about 20 min the residual current value decreased to less than 0.2 mA and no longer changed. The parameters were then set for automatic compensation of the residual current and 10.00 ml of the solution of the studied substance in methanol were added with constant bubbling with nitrogen. The end of the electrolysis after 45 to 60 min was indicated by a decrease of the current to the residual value. The charge passed was found by digital integration. Spectrophotometric and polarographic study of the coulometric reduction were carried out by sampling the solution at stages where 0, 25, 50, 75 and 100% of the substance was reduced (calculated assuming exchange of 4 electrons).

### RESULTS AND DISCUSSION

First the stability of the stock solutions in methanol was studied. The spectra of diluted solutions ( $c = 10^{-4} \text{ mol l}^{-1}$ ) were measured directly in the cuvette with a pathlength of 0.5 cm; more concentrated solutions were diluted ten-fold with methanol immediately prior to the measurement. It follows from Table I that the studied stock solutions in methanol are sufficiently stable in the dark for a period of two months. Solutions with lower concentrations were prepared fresh daily.

#### TAST POLAROGRAPHY AT A DROPPING MERCURY ELECTRODE

##### *The Effect of the pH*

Because of the limited solubility of the studied substances a methanol–Britton–Robinson buffer mixture (9 : 1) was used.

Maxima appeared on the TAST polarographic curves for all the studied substances (see Fig. 1) and complicated evaluation of the curves. As the pH increased, these maxima decreased in size and were more easily suppressed. Gelatine cannot be used for this purpose as it is not soluble in methanol. However, a solution of Triton X-100 in methanol can be used; its effect on the TAST polarogram of the chloroderivative is depicted for illustration in Fig. 1. The half-wave potential of the studied substance shifted by 10 to 20 mV to more positive values after addition of Triton X-100. Thus,

50  $\mu\text{l}$  of a 0.5% solution of Triton X-100 in methanol was added to 10 ml of polarographed solution in the TAST polarographic measurements.

TABLE I

The stability of stock solutions of *N,N*-dimethyl-4-amino-4'-haloazobenzenes in methanol

Halogen	$\lambda_{\text{max}}^a$ nm	$c$ $\text{mol l}^{-1}$	$t$ , days <sup>b</sup>			
			0	10	20	60
F	404	$10^{-3}$	100.0	99.6	97.9	100.3
		$10^{-4}$	100.0	99.5	99.0	98.8
Cl	415	$10^{-3}$	100.0	100.1	101.1	101.3
		$10^{-4}$	100.0	101.4	101.1	101.2
Br	415	$10^{-3}$	100.0	101.2	100.5	101.3
		$10^{-4}$	100.0	98.8	98.5	98.1
I	417	$10^{-3}$	100.0	101.5	99.6	100.4
		$10^{-4}$	100.0	101.5	101.2	100.3

<sup>a</sup> Wavelength of the absorption maximum at which the stability was followed; <sup>b</sup> percent related to the original concentration of the azo compound.

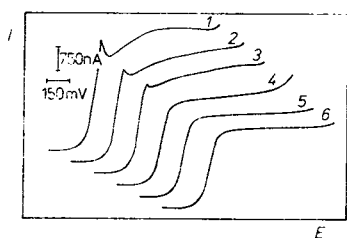


FIG. 1

The effect of Triton X-100 on the TAST polarography of *N,N*-dimethyl-4-amino-4'-chloroazobenzene ( $c = 10^{-4} \text{ mol l}^{-1}$ ) in Britton-Robinson buffer-methanol medium (1 : 9) at pH 6.7. To 10 ml of polarographed solution were added 0 (1), 10 (2), 30 (3), 50 (4), 100 (5) and 500 (6)  $\mu\text{l}$  of 0.5% Triton X-100 solution in methanol. Initial potential  $-200 \text{ mV}$

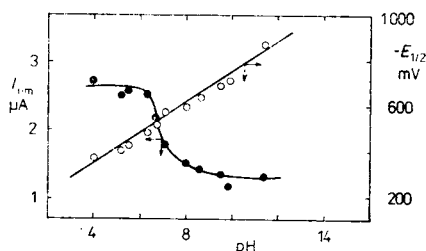


FIG. 2

The dependence of the limiting current ( $I_{\text{lim}}$ ) and the half-wave potential ( $E_{1/2}$ ) of *N,N*-dimethyl-4-amino-4'-fluoroazobenzene ( $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) on the pH in a Britton-Robinson buffer-methanol medium (1 : 9)

The determined dependences of the half-wave potential, limiting currents and slopes of the logarithmic analysis on the pH are given in Table II. For illustration, Fig. 2 depicts the dependence of the half-wave potential and limiting current on the pH for the fluoroderivative. It can be seen that the dependence of  $E_{1/2}$  on the pH is linear; the parameters for the studied substances calculated by the method of linear regressions are listed in Table III. The observed shift in  $E_{1/2}$  with decreasing pH to more positive values can be explained by preliminary protonation of the azo group, leading to a decrease in the electron density in the region of the bond between the

TABLE II

The effect of the pH on the TAST polarograms of N,N-dimethyl-4-amino-4'-haloazobenzenes ( $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) in Britton-Robinson buffer-methanol medium (1 : 9)

pH	$E_{1/2}$ mV	$I_{lim}$ $\mu\text{A}$	$\alpha^a$ mV	$E_{1/2}$ mV	$I_{lim}$ $\mu\text{A}$	$\alpha^a$ mV
	F-derivative			Cl-derivative		
4.0	-367	2.72	93	-330	2.91	79
5.2	-405	2.50	74	-403	3.16	72
5.5	-423	2.56	69	-423	2.80	66
6.3	-493	2.49	69	-476	2.52	57
6.7	-523	2.15	62	-496	2.21	53
7.1	-573	1.75	54	-539	2.19	67
8.0	-597	1.50	54	-597	2.18	65
8.6	-640	1.39	54	-637	2.10	81
9.5	-692	1.33	69	-707	2.20	124
9.9	-707	1.17	69	-730	2.01	114
11.4	-870	1.30	93	-835	2.11	87
	Br-derivative			I-derivative		
4.0	-373	2.68	137	-300	2.40	103
5.2	-385	2.71	71	-355	2.20	59
5.5	-402	2.68	66	-370	2.30	48
6.3	-445	2.42	54	-380	1.87	41
6.7	-480	2.11	51	-475	1.67	41
7.1	-530	1.91	43	-510	1.44	41
8.0	-547	1.73	40	-520	1.49	45
8.6	-603	1.74	56	-590	1.48	46
9.5	-647	1.65	59	-635	1.61	54
9.9	-685	1.51	69	-665	1.30	39
11.4	-720	1.72	117	-720	1.50	32

<sup>a</sup> Slopes of the logarithmic analyses.

two nitrogen atoms and thus simplified reduction. It can also be seen from Fig. 2 that the limiting current observed in acid medium is roughly twice as large as that in alkaline medium, which can be explained by a change in the number of exchanged electrons on transition from an acid to an alkaline solution. The results of logarithmic analysis of the TAST polarograms (see Table II) indicate that the process is irreversible; this was also confirmed by cyclic voltammetry at a hanging mercury drop electrode. No anodic peak was observed for the studied substances at pH 4–12 and polarization rate of 5–50 mV s<sup>-1</sup>; thus the system can be considered completely irreversible. It was also found<sup>22</sup> that the expression  $I_p/\sqrt{\nu}$  (where  $I_p$  is the height of the cathodic peak in cyclic voltammetry and  $\nu$  is the polarization rate) is not constant, as required by the equations for a completely irreversible process<sup>23</sup>. Thus, the electrode process is more complex and does not correspond to simple charge transfer. From an analytical point of view, the best-developed waves were found in a medium with pH 6.7; thus, all other dependences were studied in this medium. The diffusion character of the limiting currents was verified by measuring the dependence of the wave height in DC polarography on the square root of the height of the mercury reservoir and confirmed by the linear character of the dependence of the wave height on the depolarizer concentration in the range  $1 \cdot 10^{-4}$  to  $1 \cdot 10^{-6}$  mol l<sup>-1</sup>. The determined dependence of the half-wave potential of the depolarizer<sup>22</sup> on its concentration apparently is connected with the irreversibility of the studied process. The parameters of the calibration straight lines and the determination limit for the studied substances using TAST polarography are given in Table IV.

The effect of the nature of the substituent on the half-wave potential of the studied substances can be seen in Fig. 3. The measured  $E_{1/2}$  values are well correlated with the Hammett  $\sigma$  constants of the substituents, confirming that the series of substances

TABLE III

The parameters of the linear dependence of the half-wave potentials of N,N-dimethyl-4-amino-4'-haloazobenzenes on pH.  $c(\text{azo}) = 1 \cdot 10^{-4}$  mol l<sup>-1</sup>; Britton-Robinson buffer-methanol (1 : 9);  $E_{1/2} = a + b \text{ pH}$

Halogen	$a$ , mV	$b$ , mV pH <sup>-1</sup>	$r^a$
F	-77.5	-66.3	0.9965
Cl	-59.5	-67.1	0.9879
Br	-92.4	-59.2	0.9969
I	-51.3	-60.9	0.9959

<sup>a</sup> Correlation coefficient.

studied is reduced by a single mechanism. The observed correlation is especially satisfactory as deviations from linearity of the  $E_{1/2}$  vs  $\sigma$  dependence are most often

TABLE IV

The parameters of the calibration straight lines and the determination limits of N,N-dimethyl-4-amino-4'-haloazobenzenes using TAST polarography

Halogen	$c$ $\mu\text{mol l}^{-1}$	Slope $\mu\text{A mol}^{-1} \text{ l}$	Intercept $\mu\text{A}$	$r^a$	DL <sup>b</sup> $\mu\text{mol l}^{-1}$
F	10–100	$2.15 \cdot 10^4$	-0.02	0.9993	—
	1–10	$2.25 \cdot 10^4$	-0.02	0.9973	1.1
Cl	10–100	$2.21 \cdot 10^4$	-0.04	0.9980	—
	1–10	$2.31 \cdot 10^4$	-0.03	0.9952	1.5
Br	10–100	$2.11 \cdot 10^4$	-0.04	0.9993	—
	1–10	$2.21 \cdot 10^4$	0.01	0.9998	0.3
I	10–100	$1.67 \cdot 10^4$	-0.02	0.9989	—
	1–10	$1.97 \cdot 10^4$	0.01	0.9995	0.5

<sup>a</sup> Correlation coefficient; <sup>b</sup> determination limit.

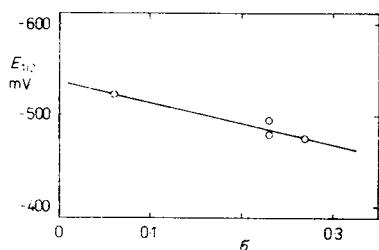


FIG. 3

The relationship between the half-wave potential ( $E_{1/2}$ ) of N,N-dimethyl-4-amino-4'-haloazobenzenes and the Hammett  $\sigma$ -constants of the substituents. Depolarizer concentration  $1 \cdot 10^{-4} \text{ mol l}^{-1}$ ; medium — Britton–Robinson buffer–methanol (1:9), pH 6.7

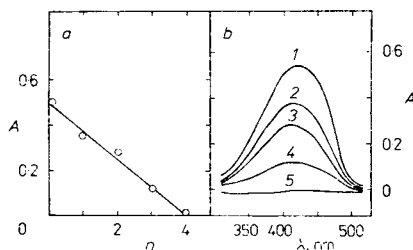


FIG. 4

Spectrophotometric study of the reduction of N,N-dimethyl-4-amino-4'-chloroazobenzene using coulometry at a constant potential of  $-750 \text{ mV}$ . *a* Dependence of the absorbance at  $415 \text{ nm}$  on the charge passed  $Q$  recalculated to the number of electrons  $n$  per molecule of reduced substance; *b* spectrum of the solution after passage of charge corresponding to  $n = 0$  (1), 1 (2), 2 (3), 3 (4), and 4 (5)

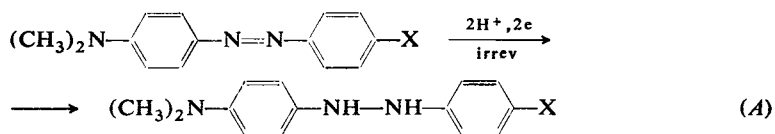
observed for haloderivatives<sup>24</sup>. The halogen atoms are easily deformed and can act as electron bridges in some electrode reactions, resulting in a change in the values of the charge transfer coefficients.

#### *Determination of the Number of Electrons Exchanged*

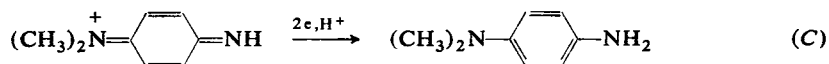
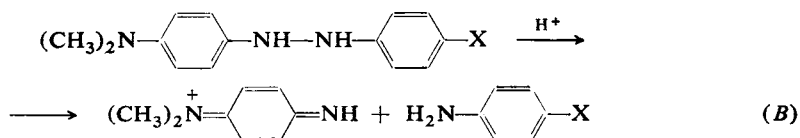
It was found from coulometry at constant potential at a large-area mercury electrode that all the studied substances in Britton–Robinson buffer–methanol solution (1 : 9) at pH 6.7 exchange four electrons at a potential of  $-750$  mV. This result was also confirmed by spectrophotometry (see Fig. 4) and TAST polarography (see Fig. 5) of the studied solutions.

#### *Proposed Mechanism of the Electrochemical Reaction*

It can be assumed on the basis of the above information that the electrode process first involves a two-electron reduction of the studied azocompounds according to Eq. (A), where X = F, Cl, Br or I:



Acid-catalyzed hydrolysis of the hydrazocompound formed occurs in acid medium according to Eq. (B) and the quinone diimine formed is immediately reduced to the corresponding amine according to Eq. (C), with a total exchange of 4 electrons. In alkaline medium, the rate of disproportionation is much lower, leading to a decrease in the height of the observed wave to a value corresponding to the exchange of two electrons.



### DIFFERENTIAL PULSE POLAROGRAPHY AT A DROPPING MERCURY ELECTRODE

#### *The Effect of the pH*

It follows from Table V and Fig. 6 that the dependence of the peak potential  $E_p$  on the pH has the same character as the dependence of  $E_{1/2}$  on the pH and can be



TABLE V

The effect of the pH on the DP polarograms of N,N-dimethyl-4-amino-4'-haloazobenzenes ( $c(\text{azo}) = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) in Britton-Robinson buffer-methanol (1 : 9)

pH	F-derivative		Cl-derivative		Br-derivative		I-derivative	
	$E_p$ mV	$I_p$ $\mu\text{A}$	$E_p$ mV	$I_p$ $\mu\text{A}$	$E_p$ mV	$I_p$ $\mu\text{A}$	$E_p$ mV	$I_p$ $\mu\text{A}$
4.0	-377	2.91	-263	4.80	-365	1.42	-340	1.79
5.2	-415	2.99	-436	3.25	-395	3.01	-370	3.39
5.5	-438	3.61	-483	3.90	-407	3.81	-390	3.39
6.3	-440	3.51	-500	4.12	-477	4.60	-430	3.61
6.7	-512	3.60	-538	4.12	-495	4.58	-475	3.93
7.1	-550	3.30	-584	3.60	-515	4.72	-510	3.90
8.0	-575	2.95	-635	3.08	-545	4.31	-535	3.71
8.6	-625	2.31	-737	1.92	-595	3.49	-590	3.68
9.5	-670	1.59	-802	2.05	-644	2.65	-640	3.00
9.9	-695	1.30	-820	2.32	-695	1.86	-665	2.61
11.4	-880	0.85	-838	3.68	-800	2.11	-725	2.69

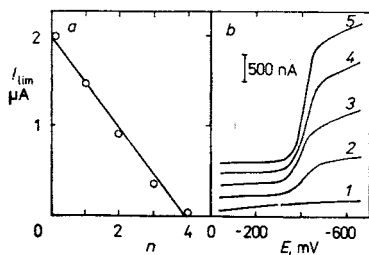


FIG. 5

TAST polarographic study of the reduction of N,N-dimethyl-4-amino-4'-chloroazobenzene using coulometry at constant potential of  $-750 \text{ mV}$ . *a* Dependence of the limiting current on the charge passed  $Q$  recalculated to the number of electrons  $n$  per molecule of reduced substance; *b* TAST polarograms of the solution after passage of charge corresponding to  $n = 0$  (5), 1 (4), 2 (3), 3 (2), and 4 (1)

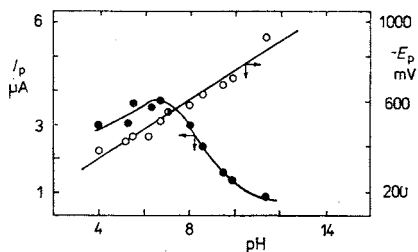


FIG. 6

Dependence of the peak height ( $I_p$ ) and peak potential ( $E_p$ ) for DPP of N,N-dimethyl-4-amino-4'-fluoroazobenzene on the pH in Britton-Robinson buffer-methanol mixture (1 : 9)

explained in the same way. The parameters of the former dependence calculated by the method of linear regressions are listed in Table VI. The dependence of the peak height  $I_p$  on the pH is apparently a combination of the effects of the pH on the number of exchanged electrons and on the reversibility of the studied process.

TABLE VI

Parameters for the dependence of the potentials of the DPP peaks of N,N-dimethyl-4-amino-4'-haloazobenzenes on the pH.  $c(\text{azo}) = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ; Britton-Robinson buffer-methanol (1 : 9);  $E_p = a + b \cdot \text{pH}$

Halogen	$a$ , mV	$b$ , mV pH <sup>-1</sup>	$r^a$
F	-73.9	-62.3	0.9846
Cl	-84.0	-69.6	0.9798
Br	-82.0	-60.6	0.9990
I	-85.1	-57.4	0.9949

<sup>a</sup> Correlation coefficient.

TABLE VII

The stability of solutions of N,N-dimethyl-4-amino-4'-haloazobenzenes in Britton-Robinson buffer-methanol medium (1 : 9) with pH 6.7

Halogen	$c$ mol l <sup>-1</sup>	$t$ , min <sup>a</sup>		
		10	15	30
F	$10^{-4}$	100.0	100.9	102.4
	$10^{-5}$	100.0	100.8	104.3
	$10^{-6}$	100.0	100.9	102.0
Cl	$10^{-4}$	100.0	101.1	102.3
	$10^{-5}$	100.0	101.3	104.0
	$10^{-6}$	100.0	100.8	102.8
Br	$10^{-4}$	100.0	101.1	102.8
	$10^{-5}$	100.0	101.1	103.2
	$10^{-6}$	100.0	100.7	101.7
I	$10^{-4}$	100.0	101.9	103.8
	$10^{-5}$	100.0	100.9	102.9
	$10^{-6}$	100.0	100.7	101.7

<sup>a</sup> Percent related to the original concentration of the azocompound.

TABLE VIII

Parameters of the calibration straight lines and determination limits for N,N-dimethyl-4-amino-4'-haloazobenzenes using differential pulse polarography

Halogen	$c$ $\mu\text{mol l}^{-1}$	Slope $\mu\text{A mol}^{-1} \text{l}$	Intercept $\mu\text{A}$	$r^a$	$\text{DL}^b$ $\mu\text{mol l}^{-1}$
F	10–100	$3.62 \cdot 10^4$	$4.2 \cdot 10^{-1}$	0.9982	—
	1–10	$7.32 \cdot 10^4$	$-1.3 \cdot 10^{-2}$	0.9975	—
	0.1–1	$1.03 \cdot 10^5$	$-1.2 \cdot 10^{-3}$	0.9996	0.05
Cl	10–100	$4.24 \cdot 10^4$	$0.8 \cdot 10^{-1}$	0.9989	—
	1–10	$8.22 \cdot 10^4$	$-1.3 \cdot 10^{-2}$	0.9990	—
	0.1–1	$1.24 \cdot 10^5$	$-1.7 \cdot 10^{-3}$	0.9992	0.06
Br	10–100	$4.57 \cdot 10^4$	$3.2 \cdot 10^{-1}$	0.9986	—
	1–10	$6.69 \cdot 10^4$	$-1.0 \cdot 10^{-2}$	0.9993	—
	0.1–1	$7.67 \cdot 10^4$	$0.9 \cdot 10^{-3}$	0.9978	0.10
I	10–100	$3.83 \cdot 10^4$	$2.0 \cdot 10^{-1}$	0.9977	—
	1–10	$5.05 \cdot 10^4$	$-0.9 \cdot 10^{-2}$	0.9999	—
	0.1–1	$6.06 \cdot 10^4$	$0.5 \cdot 10^{-3}$	0.9990	0.07

<sup>a</sup> Correlation coefficient; <sup>b</sup> determination limit.

TABLE IX

The effect of the pH on the LS voltammograms of N,N-dimethyl-4-amino-4'-haloazobenzenes ( $c(\text{azo}) = 1 \cdot 10^{-5} \text{ mol l}^{-1}$ ) in Britton–Robinson buffer–methanol medium (1 : 9)

pH	F-derivative		Cl-derivative		Br-derivative		I-derivative	
	$E_p$ mV	$I_p$ nA	$E_p$ mV	$I_p$ nA	$E_p$ mV	$I_p$ nA	$E_p$ mV	$I_p$ nA
4.0	–250	180	–190	168	–205	185	–220	148
5.2	–340	185	–310	238	–315	190	–310	205
5.5	–385	205	–340	238	–340	190	–350	228
6.3	–435	198	–390	238	–405	180	–410	228
6.7	–480	180	–425	213	–440	170	–445	200
7.1	–535	135	–470	188	–495	160	–505	185
8.0	–570	115	–540	153	–520	160	–530	180
8.6	–620	108	–570	150	–590	150	–570	175
9.5	–670	113	–610	148	–610	150	–620	183
9.9	–685	105	–640	150	–640	160	–635	173
11.4	–710	88	–675	133	–665	145	–685	140

Similarly as in TAST polarography, the best-developed peaks were again obtained at pH 6.7 and this value was employed for measuring all the other dependences.

#### *The Stability of the Polarographed Solutions*

This stability was studied in Britton–Robinson buffer–methanol solution (1 : 9) at pH 6.7. It follows from Table VII that the stability of the most dilute solution is sufficient for practical purposes. The apparent increase in concentration at longer times is apparently a result of evaporation of methanol, which could not be completely eliminated even by passing the nitrogen through pure methanol prior to passing through the polarographic vessel.

#### *Concentration Dependences*

The dependences of the peak heights on the concentration of the studied depolarizer are linear in the range  $10^{-4}$  to  $10^{-7}$  mol l<sup>-1</sup> and their parameters calculated by the method of linear regression are given in Table VIII together with the calculated determination limits. At higher concentrations (of the order of  $10^{-5}$  to  $10^{-6}$  mol l<sup>-1</sup>) the peak height was measured from the line connecting the minima on both sides of the maximum. At lower concentrations (of the order of  $10^{-7}$  mol l<sup>-1</sup>) the peak height had to be measured from the baseline for the base electrolyte which often contained peaks that coincided with those for the studied substances. (The peaks corresponding to the electrolyte could not be removed by diluting the buffer, decreasing the methanol content or one-hour pre-electrolysis of the base electrolyte on a large-area mercury electrode at a constant potential of  $-750$  mV).

#### *Linear Scan Voltammetry (LSV)*

The effect of the pH on linear scan voltammetry of the studied substances is documented by Table IX and Table X gives the parameters for the dependence of the peak potential on the pH. It can be seen that the dependence of the peak potential on the pH has the same character as in TAST and DP polarography. However, the peak height is optimal at pH 5.5 (see Fig. 7), and is best measured against the baseline for the base electrolyte. Under these conditions, the dependences of the peak height on the concentration are linear in the range  $5 \cdot 10^{-6}$  to  $10^{-7}$  mol l<sup>-1</sup> (see Table XI). Deviations from linearity were observed at depolarizer concentrations above  $5 \cdot 10^{-6}$  mol l<sup>-1</sup>, apparently as a result of passivation of the electrode by products of the electrode reaction.

The sensitivity of this technique was increased by accumulation of the determined substance by adsorption on the surface of a hanging mercury drop. It followed from measurement of the electrocapillary curves in the presence and absence of the studied substance<sup>22</sup> that the depolarizer is adsorbed on the surface of the mercury drop electrode in the potential range from 0 to  $-0.4$  V, appearing as a decrease in the

surface tension. This adsorption leads to an increase in the LSV peak at the HMDE with time elapsed from formation of the drop up to recording of the voltammetric curve; this growth is increased by stirring the solution. It can be seen from Fig. 8 that this increase is greater at lower methanol concentrations. A 120-second accumulation was found to be optimal in stirred Britton–Robinson buffer–methanol solution (1 : 1) for optimal height and shape of the voltammogram obtained and for optimal shape of the curve for the base electrolyte. Under these conditions, the dependences of the peak height on the concentration are linear in the range  $5 \cdot 10^{-7}$

TABLE X

Parameters of the dependence of the potentials of the LSV peaks of N,N-dimethyl-4-amino-4'-haloazobenzenes on the pH.  $c(\text{azo}) = 1 \cdot 10^{-5} \text{ mol l}^{-1}$ , Britton–Robinson buffer–methanol (1 : 9);  $E_p = a + b \text{ pH}$

Halogen	$a$ , mV	$b$ , mV $\text{pH}^{-1}$	$r^a$
F	-20.1	-66.3	0.9839
Cl	86.9	-66.8	0.9825
Br	7.7	-64.6	0.9798
I	2.1	-64.5	0.9829

<sup>a</sup> Correlation coefficient.

TABLE XI

Parameters of the calibration straight lines and determination limits for N,N-dimethyl-4-amino-4'-haloazobenzenes for LSV at the HMDE

Halogen	$c$ $\mu\text{mol l}^{-1}$	Slope $\mu\text{A mol}^{-1} \text{ l}$	Intercept $\mu\text{A}$	$r^a$	DL <sup>b</sup> $\mu\text{mol l}^{-1}$
F	1–10	$2.12 \cdot 10^4$	0.009	0.9995	—
	0.1–1	$3.07 \cdot 10^4$	0.001	0.9999	0.07
Cl	1–10	$2.31 \cdot 10^4$	0.024	0.9916	—
	0.1–1	$3.52 \cdot 10^4$	0.001	0.9992	0.08
Br	1–10	$1.97 \cdot 10^4$	0.020	0.9914	—
	0.1–1	$3.72 \cdot 10^4$	0.030	0.9981	0.05
I	1–10	$2.27 \cdot 10^4$	0.019	0.9971	—
	0.1–1	$3.87 \cdot 10^4$	0.003	0.9998	0.07

<sup>a</sup> Correlation coefficient; <sup>b</sup> determination limit.

to  $2 \cdot 10^{-8} \text{ mol l}^{-1}$ . Deviations from linearity occur at higher concentrations, apparently as a result of maximal permissible coverage of the drop surface by the adsorbed substance. The parameters of the calibration straight lines and calculated determination limits are given in Table XII.

The newly developed methods for the determination of the 4-haloderivatives of N,N-dimethyl-4-aminoazobenzene, especially differential pulse polarography and linear scan voltammetry at a hanging mercury drop, are much more sensitive than the spectrophotometric determination. In differential pulse polarography under

TABLE XII

The parameters of the calibration straight lines and determination limits of N,N-dimethyl-4-amino-4'-haloazobenzenes using LSV at the HMDE and adsorptive accumulation

Halogen	$c$ $\mu\text{mol l}^{-1}$	Slope $\mu\text{A mol}^{-1} \text{ l}$	Intercept $\mu\text{A}$	$r^a$	$\text{DL}^b$ $\text{nmol l}^{-1}$
F	0.01–0.1	$4.05 \cdot 10^5$	$4.2 \cdot 10^{-3}$	0.9918	9
Cl	0.01–0.1	$4.35 \cdot 10^5$	$4.8 \cdot 10^{-3}$	0.9898	11
Br	0.01–0.1	$4.73 \cdot 10^5$	$5.1 \cdot 10^{-3}$	0.9910	13
I	0.01–0.1	$4.91 \cdot 10^5$	$3.9 \cdot 10^{-3}$	0.9921	10

<sup>a</sup> Correlation coefficient; <sup>b</sup> determination limit.

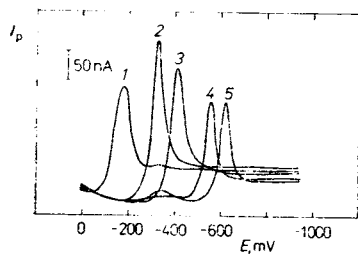


FIG. 7

The effect of the pH on the LSV voltammogram of N,N-dimethyl-4-amino-4'-chloroazobenzene ( $c = 1 \cdot 10^{-5} \text{ mol l}^{-1}$ ) in Britton–Robinson buffer–methanol medium (1:9) with pH 4.0 (1), 5.5 (2), 6.7 (3), 8.6 (4) and 9.9 (5)

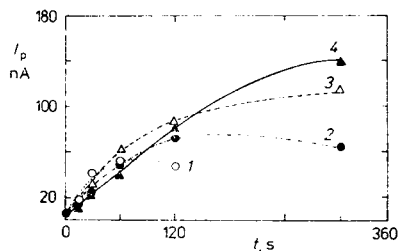


FIG. 8

Dependence of the height of LSV peak of N,N-dimethyl-4-amino-4'-chloroazobenzene ( $c = 2 \cdot 10^{-7} \text{ mol l}^{-1}$ ) on the accumulation time and methanol content in medium with pH 5.5. Accumulation carried out in stirred solution, recorded 10 s after termination of stirring. Methanol content 90 (1), 70 (2), 50 (3), and 30 (4) vol. %

suitable conditions, the difference in the peak potentials for some pairs of studied substances is up to 50 mV (for example, the 4-fluoro- and 4-iododerivatives at pH 6.3), so that this technique will apparently be useful for analysis of a mixture of these two substances, especially when the evaluation is carried out on the basis of mathematical analysis of the curves on a computer<sup>25</sup>. Spectrophotometry in the visible region cannot be used for this purpose as the maxima of the studied substances are practically identical (see Tab. 1). Substances whose peak potentials are not sufficiently different must be analyzed using preliminary separation by thin-layer chromatography<sup>16</sup>.

## REFERENCES

1. Miller J., Miller E. C., Finger G. C.: *Cancer Res.* 17, 387 (1975).
2. Hansch C., Fujita C.: *J. Am. Chem. Soc.* 86, 1616 (1984).
3. Egan H. (Ed.): *Environmental Carcinogens — Selected Methods of Analysis*. Vol. 4. *Some Aromatic Amines and Azo Dyes in the General and Industrial Environment*, p. 4. IARC Scientific Publications No. 40. International Agency for Research on Cancer, Lyon 1981.
4. Barek J. in: *Nové směry v analytické chemii IV* (Zýka J., Ed.), p. 147. SNTL, Prague 1988.
5. Barek J., Kelnar L.: *Collect. Czech. Chem. Commun.* 50, 712 (1985).
6. Barek J., Hrnčír R.: *Collect. Czech. Chem. Commun.* 51, 25 (1986).
7. Barek J., Hrnčír R.: *Collect. Czech. Chem. Commun.* 51, 2083 (1986).
8. Barek J., Pastor T. J., Votavová S., Zima J.: *Collect. Czech. Chem. Commun.* 52, 2149 (1987).
9. Barek J., Hrnčír R.: *Microchem. J.* 36, 172 (1987).
10. Fogg A. G., Yoo K. S.: *Analyst* 104, 723 (1979).
11. Hart J. P., Smyth W. F.: *Analyst* 105, 938 (1980).
12. Bond A. M.: *Modern Polarographic Methods in Analytical Chemistry*. M. Dekker, New York 1980.
13. Stradyns J., Glezer V. in: *Encyclopedia of the Electrochemistry of the Elements* (Bard A. J. and Lund H., Eds), Vol. 13, p. 163. M. Dekker, New York 1979.
14. Thomas F. G., Botto K. G. in: *The Chemistry of the Hydrazo, Azoxy and Azocompounds* (Patai S., Ed), p. 443. J. Wiley, Chichester 1975.
15. Mairanovski S. G., Stradyns J. P., Bezuglyi V. V.: *Polarografiya v organicheskoi khimii*, p. 218. Khimiya, Leningrad 1975.
16. Barek J., Berka A., Borek V.: *Microchem. J.* 27, 229 (1982).
17. Burcinová A., Štulík K., Pacáková V.: *J. Chromatography* 389, 397 (1987).
18. Barek J., Berka A., Borek V.: *Collect. Czech. Chem. Commun.* 47, 495 (1982).
19. Sýkora V., Zátka V.: *Příruční tabulky pro chemiky*, 3rd. Ed., p. 66. SNTL, Prague 1967.
20. De Ligny C. Z., Luykx P. F. M., Rehrbach M., Wienecke A. A.: *Rec. Trav. Chim.* 79, 713 (1960).
21. Skogerboe R. K., Grant L. C.: *Spectrosc. Lett.* 3, 215 (1970).
22. Haladová-Bláhová H.: *Thesis*. Charles University, Prague 1988.
23. Greef R., Peat R., Peter L. M., Pletcher D., Robinson J.: *Instrumental Methods in Electrochemistry*, p. 178. Ellis Horwood, Chichester 1985.
24. Zuman P.: *Vplyv substituentov v organickej polarografii*, p. 47. Alfa, Bratislava 1970.
25. Zimová N., Němec I., Zima J.: *Talanta* 33, 467 (1986).

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