POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF *N*,*N*-DIMETHYL-4-AMINO-4′-SULFOAZOBENZENE

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The polarographic behaviour of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene was investigated by tast polarography and differential pulse polarography at a dropping mercury electrode, constant-potential coulometry at a large area mercury electrode and cyclic voltammetry at a hanging mercury drop electrode. Optimum conditions have been found for its determination by tast polarography in the concentration range of 2–100 μmol l^{-1}, differential pulse polarography at a dropping mercury electrode in the concentration range of 0.2–100 μmol l^{-1} and by differential pulse voltammetry at a hanging mercury drop electrode in the concentration range of 0.02–10 μmol l^{-1}. The sensitivity of the determination can be further improved by adsorptive accumulation of the test substance on the hanging mercury drop electrode. Three-minute accumulation in stirred solution allows determination in the range of 0.2–100 nmol l^{-1}.

Key words: Polarography; Voltammetry; *N*,*N*-Dimethyl-4-amino-4′-sulfoazobenzene.

Derivatives of *N*,*N*-dimethyl-4-aminoazobenzene are among the best known genotoxic azo compounds[1] and are often used as model substances in various toxicological studies. It follows from QSAR correlation between the biological activity and the structure of *N*,*N*-dimethyl-4-aminoazobenzene derivatives that their carcinogenity generally increases with incresing lipophility[2]. However, newer sources[3,4] have pointed out that even the more polar substance, *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene is mutagenic. Even very small amounts of mutagenic substances can have a detrimental effect on biological processes and thus a great deal of attention has been paid to analytical methods for the determination of trace amounts of these substances in both biological samples and the environment[5]. The easy polarographic reduction of azo compounds, whose mechanism is discussed in monographs[6,7], permits the very sensitive determination of a number of genotoxic derivatives of *N*,*N*-dimethyl-4-aminoazobenzene[8–10] using modern techniques such as differential pulse polarography (DPP) at a classical

dropping mercury electrode (DME), differential pulse voltammetry (DPV) at a hanging mercury drop electrode (HMDE) or adsorptive stripping voltammetry (AdSV).

Determination of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene using classical DC polarography[11,12], AC polarography[13] and oscillopolarography[14] was described earlier. The DC polarography was used for a detailed study of the adsorption of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene at a classical dropping mercury electrode[15,16] and the interaction of this substance with bovine serum albumin[17,18]. The DC or AC polarography, however, is not sensitive enough for the determination of micromolar and nanomolar concentrations of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene. Consequently, this work is concerned with the use of tast polarography, DPP, DPV and AdSV for the determination of trace amounts of this genotoxic azo compound.

EXPERIMENTAL

Reagents

The stock solution of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene (c = 1 mmol l^{-1}) was prepared by dissolving 0.03273 g of the substance (Sigma) in 100 ml of deionized water. The purity of the substance was controlled by TLC (ref.[19]) and HPLC (ref.[20]). More diluted solutions were prepared by exact dilution of the stock solution. All the solutions were stored in the dark. It followed from a spectrophotometric study of the stability of these solutions[21] that the solution with a concentration of 1 mmol l^{-1} must be prepared fresh once a month, 0.1 mmol l^{-1} every week and 0.01 mmol l^{-1} daily. The other chemicals employed were of analytical grade purity (Lachema Brno, Czech Republic). Britton–Robinson buffers were prepared in a usual way[22]. Deionized water was produced by a Milli-Q_{plus} system.

Apparatus

A PA 3 polarographic analyzer was employed together with an XY 4106 *x*–*y* recorder (both from Laboratorni pristroje, Prague, Czech Republic). Measurements were carried out using a three-electrode arrangement with a platinum wire auxiliary electrode and saturated silver chloride reference electrode, to which all potential values are referred. Parameters of the classical DME used in tast and DP polarography were as follows: At a mercury reservoir height of h = 64 cm, the flow rate was m = 1.453 mg s^{-1} and the drop time τ = 5.3 s (at an applied potential of 0 V in 0.1 M KCl). Where not stated otherwise, experiments were carried out at a sweep rate of 5 mV s^{-1}, controlled drop time of 1 s, mercury reservoir height of 64 cm and modulation amplitude in differential pulse polarography of –100 mV.

The DPV, AdSV and cyclic voltammetric measurements were carried out using a static mercury drop electrode SMDE (Laboratorni pristroje, Prague, Czech Republic) connected as a hanging mercury drop electrode (HMDE). The capillary had a diameter of 0.146 mm with the maximum attainable drop size obtained by opening the valve for 160 ms. Where not stated otherwise, measurements on HMDE were carried out at a sweep rate of 20 mV s^{-1} and the DPV modulation amplitude of 50 mV. Oxygen was removed by bubbling solutions for five minutes with nitrogen purified by passing through a solution of chromium(II) in dilute hydrochloric acid over zinc amalgam.

Coulometric measurements were performed using an OH 404 coulometric analyzer (Radelkis, Budapest, Hungary) in a 200 ml vessel. A mercury pool at the vessel bottom served as a cathode, a

platinum sheet electrode served as an anode. The cathode and anode compartments were separated with a frit. The saturated calomel electrode OH 993 (Radelkis, Budapest, Hungary) was used. The solution was stirred with a magnetic stirrer during the electrolysis, and the measurements were conducted under nitrogen.

Spectrophotometric measurements were performed using a PU 8 800 spectrophotometer (Pye Unicam, United Kingdom) and 1 cm quartz cells.

The solution pH was measured using a PHM 62 digital pH meter (Radiometer, Copenhagen, Denmark) and a glass pH and saturated calomel reference electrode.

All the measurements were carried out at laboratory temperature.

Procedures

The calibration curves were measured in triplicate and evaluated by the least-squares linear regression method. The limit of determination was calculated as the tenfold standard deviation from 7 analyte determinations at the concentration corresponding to the lowest point on the appropriate calibration straight line[23].

The procedure for the determination of the number of exchanged electrons employed constant potential coulometry at a large area mercury electrode in 90 ml of Britton–Robinson buffer, pH 4.0 or 12.0. A coulometric vessel filled with the solution was bubbled by nitrogen and stirred. Simultaneously, pre-electrolysis was commenced at a selected potential (–0.6 V at pH 4.0 or –1.2 V at pH 12.0). After about 20 min, the value of residual current decreased below 0.1 mA and no longer changed. Then the appropriate circuit parameters were adjusted for automatic compensation of the residual current and 10.00 ml of the solution of 1 mmol l^{-1} *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene, previously deoxygenated, were added under forced convection conditions (constant bubbling and stirring). Electrolysis was terminated when the value of residual current was reached (*ca* after 90 min), digital integration of the current passed yielded corresponding charge. The course of the reduction was monitored spectrophotometrically and polarographically by taking 10 ml of solution from the coulometric vessel at the given time intervals and measuring tast polarogram and UV and visible spectrum. The sampling was carried before start of coulometry and after reduction of 25, 50, 75 and 100% of the substance (calculated relative to the determined number of electrons exchanged at the constant potential). The following procedure was used for TLC monitoring of the coulometric reduction: After coulometric reduction at pH 4, the solution was neutralized with 0.2 M NaOH to pH 7, extracted in two successive steps with 10 ml of chloroform, both extracts were mixed and evaporated to 1 ml volume under a rotating vacuum evaporator. Then 30 μl of the solution was analyzed by thin-layer chromatography using a Silufol UV 254 layer and developed by the ascending method using methanol as a mobile phase. Detection was carried out by selective detection reagent for primary aromatic amines (1% of *p*-dimethylaminobenzaldehyde in ethanol–concentrated hydrochloric acid 95 : 5).

For the extraction-polarographic determination of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene 10.00 ml of the solution containing 1–10 μmol l^{-1} of the test substance were measured in a separatory funnel; 10 ml of Britton–Robinson buffer, pH 2.9, were added and the solution was extracted three times with 10 ml of butanol. Combined extracts were evaporated and dried at 70 °C using a rotating vacuum evaporator. The residue was dissolved in 10.00 ml of Britton–Robinson buffer, pH 12, and the tast or DP polarograms were recorded.

RESULTS AND DISCUSSION

Study of the Polarographic Behaviour of N,N-Dimethyl-4-amino-4′-sulfoazobenzene

Tast polarography. The dependence of a half-wave potential $E_{1/2}$, limiting current I_{lim} and the slope α of the log-plot on the pH is given in Table I. The method of linear regression yielded the relationship $E_{1/2}$ = –61.8 pH + 57.3 (in mV, correlation coefficient 0.9946) over the region of pH 2–12. The observed shift of $E_{1/2}$ to more positive values with decreasing pH can be explained by prior protonation, leading to a decrease of the electron density in the region of azo group, thus facilitating the reduction. The slopes yielded by the semilogarithmic analysis of tast polarographic curves (see Table I) indicate that these waves correspond to irreversible processes. This fact was further confirmed by the cyclic voltammetry on a hanging mercury drop electrode as described below. It follows from the slope of $E_{1/2}$ *vs* pH dependence that the same number of protons and electrons are exchanged in the process. At pH 12, the limiting current of the first wave measured by DC polarography is directly proportional to the square root of the height of mercury column confirming a diffusion-controlled process. If follows from the dependence of the limiting current on pH (Fig. 1) that twice as many electrons are exchanged in the acidic region compared to the alkaline region. This fact was further confirmed by constant potential coulometry experiments.

Constant potential coulometry. Potentiostatic coulometry at a large-area mercury electrode yielded the number of exchanged electrons n = 2.9 at pH 4 and a potential of –0.6 V or n = 2.03 at pH 12 and a potential of –1.2 V as shown in Fig. 2. Tast polarographic monitoring of the coulometric reduction under these conditions revealed the disappearance of the polarographically active azo group. Spectrophotometry performed during the coulometric reduction showed the disappearance of the absorption maximum

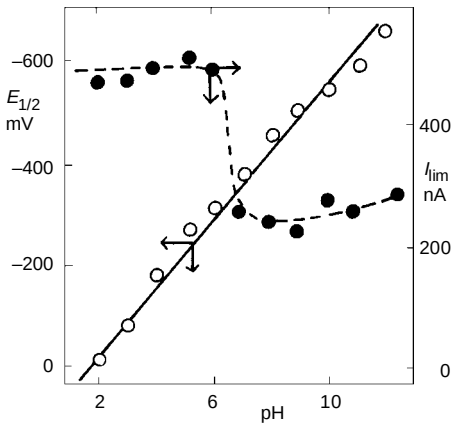


FIG. 1
The dependence of the limiting current I_{lim} and the half-wave potential $E_{1/2}$ of *N,N*-dimethyl-4-amino-4′-sulfoazobenzene (c = 0.1 mmol l^{-1}) on pH

at 460 nm ascribed to the azo group and an increase of absorbance around 240 nm which corresponds to aromatic amines or hydrazines.

An attempt was also made to identify the products of the coulometric reduction at pH 4 using thin-layer chromatography and the procedure described under Experimental. The chromatogram developed using *p*-dimethylaminobenzaldehyde contained three yellow spots with R_F values 0.35, 0.43, and 0.50, respectively. It proves that beside the two

TABLE I
The effect of pH on tast polarograms of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene, c = 0.1 mmol l^{-1}

pH	$E_{1/2}$, mV	I_{lim}, nA	α^a, mV
1.97	–25	480	57.7
3.04	–95	480	72.4
3.97	–185	500	61.9
5.04	–275	515	78.1
5.93	–315	490	74.1
6.93	–385	260	61.9
8.01	–460	250	42.9
8.98	–500	220	48.5
10.02	–545	285	37.7
10.99	–595	260	44.6
11.95	–655	310	43.0

[a] Slope of semilogarithmic analysis.

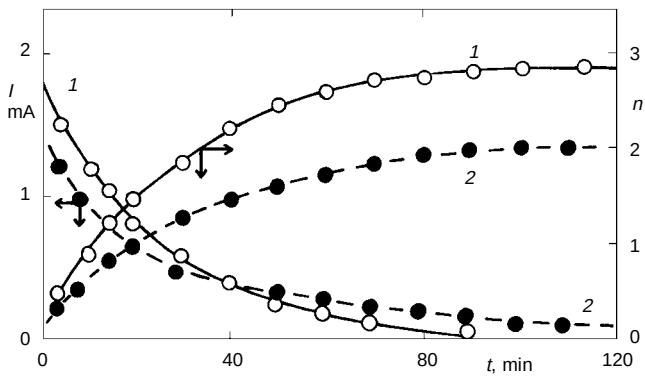
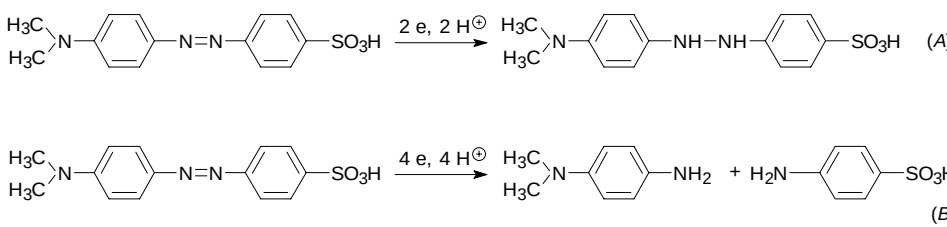


FIG. 2
Dependence of current I and calculated number of electrons n, exchanged per 1 molecule, on time t, during the coulometric reduction of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene (c = 0.1 mmol l^{-1}) in Britton–Robinson buffer. *1* pH 4 and potential –0.6 V; *2* pH 12 and potential –1.2 V

aromatic amines formed by the reduction splitting of the azo group, further primary aromatic amines are formed under these conditions, probably *via* semidine rearrangement of the intermediate hydrazo compound.

Cyclic voltammetry at a hanging mercury drop electrode. Cyclic voltammogram of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene in Britton–Robinson buffer at pH 4 and 7 demonstrates the irreversible character of the observed process; at higher pH the process becomes quasi-reversible (Fig. 3). The height of the cathodic peak is not directly proportional either to the polarization rate or to its square root, and it can be considered as a complex process controlled both by the diffusion of the test substance and by its adsorption on the electrode surface.

Proposed reduction mechanism. It can be assumed on the basis of the above observations that *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene undergoes two-electron quasi-reversible reduction at pH 12 as described by Eq. (*A*). At pH 4, the four-electron, irreversible reduction according to Eq. (*B*) can be expected. The lower number of exchanged electrons $n = 2.9$ found by constant potential coulometry at pH 4 and a potential of –0.6 V can be explained by semidine rearrangement of intermediate hydrazo compound which leads to a lower *n* than theoretically expected.



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

Tast polarography at a dropping mercury electrode. From an analytical point of view, the best developed and most easily measured curves were obtained at pH 12, which solutions are sufficiantly stable. The height of the wave of 0.1 mmol l^{-1} and 0.01 mmol l^{-1} *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene does not change after 60 min within an experimental error. In the case of 0.002 mmol l^{-1} solution the height of the wave decreases by 5% after one hour. The calibration plots are linear in the range of 2–100 μmol l^{-1} and their parameters are given in Table II.

Differential pulse polarography (DPP) at a dropping mercury electrode. *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene yields one peak with peak potential E_p and peak height I_p dependent on pH as shown in Fig. 4. The regression yielded the relationship $E_p = -59.45\ \text{pH} + 92.2$ (in mV, correlation coefficient 0.9937) over the region of pH 2–12. The highest, best developed, most readily evaluated and stable peak was

obtained in Britton–Robinson buffer at pH 12 (Fig. 5). Simultaneously, no change of I_p was observed after 30 min within an experimental error for 0.2 μmol l^{-1} *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene. Nevertheless, the polarographic curves at analyte concentration bellow 1 μmol l^{-1} should be recorded soon after preparation of the solution, preferably after a constant time period. The calibration curves are linear under these conditions in the range of 0.2–100 μmol l^{-1} and their parameters are given in Table II. The peak height was measured from the straight line connecting the minima on both sides of the peak.

Differential pulse voltammetry (DPV) at a hanging mercury drop electrode. The effect of pH on the differential pulse voltammograms of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene is documented by Fig. 6. The method of linear regression yielded the relationship E_p = –59.26 pH + 91.9 (in mV, correlation coefficient 0.9968) over the

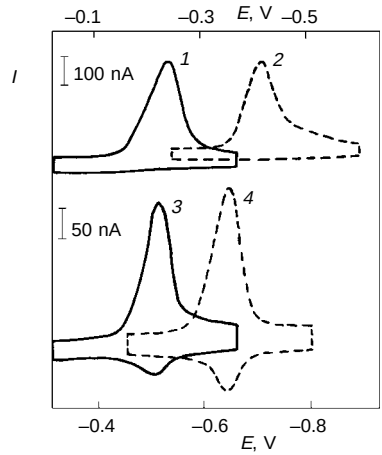


FIG. 3
Cyclic voltammogram of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene (c = 10 μmol l^{-1}) in Britton–Robinson buffer at pH 4 (*1*), 7 (*2*), 9 (*3*) and 12 (*4*) at polarization rate of 50 mV s^{-1}

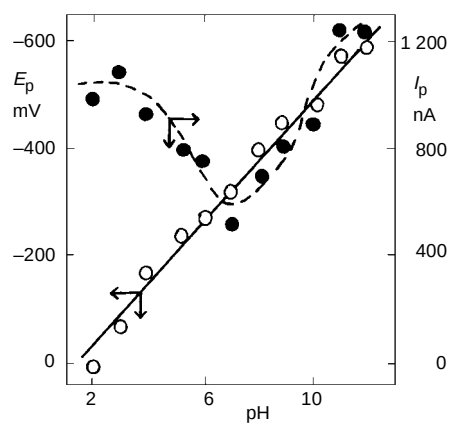


FIG. 4
The dependence of peak current I_p and peak potential E_p of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene (c = 0.1 mmol l^{-1}) on pH measured by DPP at DME

region of pH 2–12. The highest, best developed and most easily evaluated peak was obtained at pH 9 (Fig. 7). The observed splitting of the peak is probably connected with the adsorption of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene on the surface of a hanging mercury drop electrode which is not renewed during the recording of the voltammo-

TABLE II
Parameters of the calibration straight lines for the determination of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene by various polarographic and voltammetric methods in Britton–Robinson buffer

Method	pH	*c*, μmol l^{-1}	Slope mA mol^{-1} l	Intercept, nA	Correlation coefficient	L_Q[a], μmol l^{-1}
Tast	12	20–100	2.96	–13.8	0.9911	–
		2–10	2.87	–2.8	0.9897	2
DPP/DME	12	20–100	13.03	119.5	0.9985	–
		2–10	12.06	–1.3	0.9987	–
		0.2–1	11.87	0.6	0.9884	0.2
DPV/HMDE	9	2–10	143.2	–119	0.9995	–
		0.2–1	146.5	4.1	0.9992	–
		0.02–0.1	145.3	0.2	0.9965	0.02
AdSV/HMDE	9[b]	0.02–0.1	3 710	25	0.9966	–
		0.002–0.01[c]	4 620	1.5	0.9994	–
		0.0002–0.001[c]	6 100	0.3	0.9983	0.0003
	4[b]	0.02–0.1	1 339	12.3	0.9952	–
		0.002–0.01[c]	1 750	–1.2	0.9993	0.001

[a] Limit of determination; [b] 3 min accumulation in stirred solution; [c] hundred-fold diluted Britton–Robinson buffer.

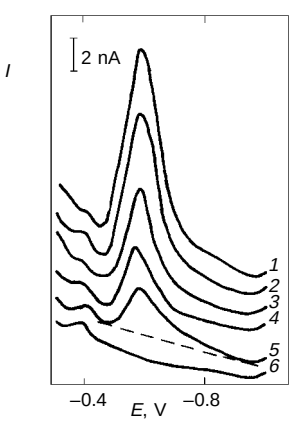


FIG. 5
DP polarograms at DME of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene at various concentrations *c* (μmol l^{-1}): *1* 1; *2* 0.8; *3* 0.6; *4* 0.4; *5* 0.2; *6* 0 at pH 12. The dashed line corresponds to the baseline from which the peak height was measured

gram. The height of the first peak was measured from the straight line connecting the minima before the first peak and after the second peak (Fig. 7). The calibration curves are linear within the concentration range of 0.02–10 μmol l^{-1} and their parameters are given in Table II.

Adsorptive stripping voltammetry (AdSV) at a hanging mercury drop electrode. A further increase of the sensitivity of determination can be attained by adsorptive accumulation of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene on the surface of a hanging mercury drop electrode. The observed dependence of the peak height I_p on the time t elapsed between the formation of the mercury drop and recording of the voltammogram is given in Table III. Measurement was performed in Britton–Robinson buffer at pH 4 and 9 at the analyte concentration of 0.2 μmol l^{-1}. A decrease of the peak current observed at accumulation times longer than 180 s in a stirred solution can be explained by reaching maximum surface coverage and by the passivation of the electrode whose

TABLE III

The effect of the accumulation time t on the peak height I_p of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene, c = 0.2 μmol l^{-1}, in Britton–Robinson buffer at pH 9, E_{acc} = –0.2 V and at pH 4 and E_{acc} = +0.1 V

t, s	0	30	60	120	180	240	300	360	420	480
I_p, nA[a]	12	13	18	24	31	37	42	49	55	64
I_p, nA[b]	12	82	170	360	465	440	375	295	230	150
I_p, nA[c]	8	12	17	24	34	42	51	57	63	69
I_p, nA[d]	8	66	118	195	233	235	227	201	180	145

[a] Accumulation at pH 9 without stirring; [b] accumulation at pH 9 with stirring; [c] accumulation at pH 4 without stirring; [d] accumulation at pH 4 with stirring.

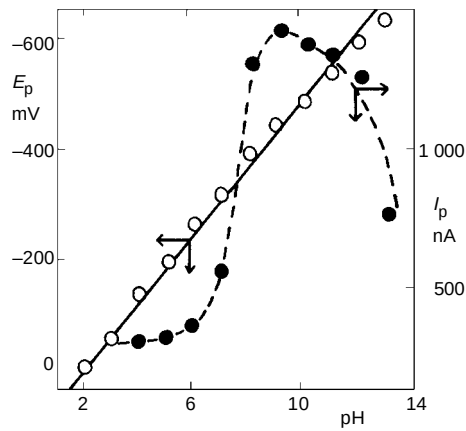


FIG. 6

The dependence of peak current I_p and peak potential E_p of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene (c = 0.01 mmol l^{-1}) on pH measured by DPV at HMDE

surface is not renewed during the measurement. Therefore, the following optimum conditions were chosen for AdSV determination of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene: Accumulation potential E_{acc} = –0.2 V, accumulation time t_{acc} = 180 s, stirred solution of Britton–Robinson buffer, pH 9 or E_{acc} = +0.1 V, t_{acc} = 180 s, stirred solution of Britton–Robinson buffer, pH 4. At the end of the accumulation period in a stirred solution, the stirrer was switched off and the differential pulse voltammogram was recorded after 15 s period to allow the solution to become quiescent. For the determination of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene at concentrations below 10 nmol l^{-1}, it is necessary to use a hundred-fold diluted Britton–Robinson buffer which yields a smoother baseline due to lower concentrations of trace impurities. The peak height was measured from the straight line connecting the minima on both sides of the peak and the value corresponding to the supporting electrolyte was subtracted. The peak height was found to be linearly dependent on the analyte concentration in the range of 0.2–100 nmol l^{-1}. The voltammograms obtained at the lowest attainable concentration range are depicted in Fig. 8. At pH 12, the splitting of the peak can be observed, especially within the concentration range of 0.02–0.1 μmol l^{-1}. It is possible, however, to carry out the determination even within the concentration range of 0.2–1 nmol l^{-1}. At pH 4, the voltammograms are better developed but they are situated at end of potential window of the supporting electrolyte which prevents from determination of subnanomolar concentration. The calibration straight line parameters are given in Table II. The limit of determination cannot be further lowered by extending the accumulation time or diluting the Britton–Robinson buffer.

Extractive Polarographic Determination of N,N-Dimethyl-4-amino-4′-sulfoazobenzene

To improve the selectivity of the developed method, combination with extraction was also studied. The study of extraction as a separation or preconcentration method was

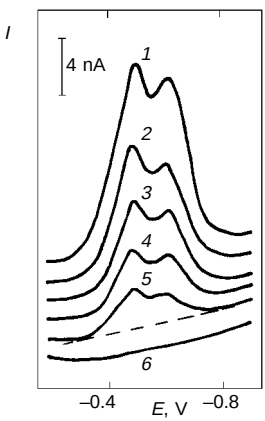


FIG. 7
DP voltammograms at HMDE of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene at various concentrations *c* (μmol l^{-1}): *1* 0.1; *2* 0.08; *3* 0.06; *4* 0.04; *5* 0.02; *6* 0 at pH 9. The dashed line corresponds to the baseline from which the peak height was measured

begun by determining the value of the distribution ratio between various organic solvents and aqueous phase at different pH. It follows from Table IV that optimum solvent for extraction is butanol and optimum pH of aqueous phase is 2.9. It was verified that using the procedure described in Experimental the recovery is 96.2%. The determination is limited to the concentration range in which tast or differential pulse polarography at dropping mercury electrode is applicable. The calibration curve should be constructed by means of standard solutions of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene employing identical procedure, *i.e.* extraction, evaporation and dissolution of the residue in Britton–Robinson buffer. Another possibility is to use less time consuming standard addition method. Again, the sample solution after addition of standard should

TABLE IV

Distribution ratios of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene between aqueous solutions with different pH and various organic solvent. Initial concentration of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene in aqueous solutions was 50 μmol l^{-1}

Solvent	pH of aqueous phase			
	1.9	2.9	4.6	7.5
Benzene	0.03	0.10	0.07	0.06
Chloroform	0.09	0.15	0.07	0.06
Ether	0.07	0.13	0.07	0.06
Butanol	9.53	16.86	14.38	13.49
Isoamylalcohol	2.22	2.60	1.74	2.29

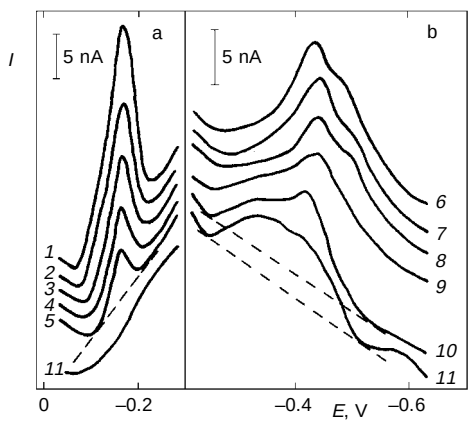


FIG. 8

DP voltammograms at HMDE of *N*,*N*-dimethyl-4-amino-4′-sulfoazobenzene in a hundred-fold diluted Britton–Robinson buffer after 180 s of accumulation at potential *E* in stirred solution at pH 4, *E* = +0.1 V (a) and pH 9, *E* = –0.2 V (b). Concentration *c* (nmol l^{-1}): *1* 10; *2* 8; *3* 6; *4* 4; *5* 2; *6* 1; *7* 0.8; *8* 0.6; *9* 0.4; *10* 0.2; *11* 0. The dashed line corresponds to the baseline from which the peak height was measured

be extracted, evaporated and the residue dissolved in Britton–Robinson buffer. The results obtained with voltammetric techniques using hanging mercury drop electrode are lower apparently due to the presence of traces of surface active substances in the solvent which was used for the extraction. To remove this interference, a combination of extraction with TLC separation would be required.

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