POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF N,N-DIMETHYL-4-AMINOAZOBENZENE*

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Conditions were found for the determination of chemical carcinogen N,N-dimethyl-4-aminoazobenzene by TAST and differential pulse polarography, fast scan differential pulse voltammetry and linear sweep voltammetry at a hanging mercury drop electrode in a mixed aqueous–methanolic medium. The detection limit of the last two methods, approximately 10^{-8} mol l^{-1}, can be further lowered by preliminary accumulation of the substance to be determined by adsorption at a working electrode. The applicability of these methods to the analysis of biological materials, directly or combined with an extraction, was demonstrated.

In a systematic study of the redox reactions of carcinogenic azo compounds, the polarographic behaviour of N,N-dimethyl-4-aminoazobenzene, a recognized carcinogen[1-3], was investigated in detail. The polarographic behaviour of azo compounds has been reviewed in the literature[4-6], and the application of the differential pulse techniques for the determination of low concentrations of azo compounds in biological materials was discussed in a previous paper[7]. In an effort to attain maximum sensitivity, differential pulse polarography (DPP) was employed at a classical dropping mercury electrode (D.M.E.) and at a static mercury dropping electrode (S.M.D.E.). When combined with the TAST technique, this led to a considerable increase in the sensitivity[8]. The other methods used were fast scan differential pulse voltammetry (FSDPV)[9] and linear sweep voltammetry (LSV) at a hanging mercury drop electrode (H.M.D.E.) which, after preconcentration of the substance at the working electrode[10], led to a further increase in the sensitivity.

EXPERIMENTAL

Reagents

The stock solution of N,N-dimethyl-4-aminoazobenzene in methanol or in chloroform (1 mmol . l^{-1}) was prepared by dissolving an exactly weighed amount of the substance (Research Institute for Organic Synthesis, Pardubice-Rybitví); the low concentration solutions were prepared by exact dilution of the stock solution with the given solvent. A 50 μmol l^{-1} solution of the studied

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substance in 0·4 mol l^{-1} hydrochloric acid was prepared by ultrasonic dissolution. The ammonia and Britton–Robinson buffer solutions were prepared conventionally[11] and checked with a digital pH-meter. All chemicals and solvents used were of reagent grade purity. The solvents were freshly redistilled before use. Water was doubly distilled in a quartz apparatus.

Apparatus

The AC polarograms were obtained using an OH 105 (Radelkis) apparatus in a three-electrode arrangement (D.M.E., S.C.E., Pt auxiliary electrode) with electronically controlled drop time (1 s) and a mercury column height of 49 cm. The polarization rate was 400 mV min^{-1} with an amplitude of 10 mV. The TAST, DPP, FSDPV, and LSV recordings were made on a PA 3 apparatus interfaced to an XY-4105 recorder (Laboratorní přístroje, Prague), also with a three electrode arrangement. Unless otherwise stated, the measurements were performed using a polarization rate of 2 mV s^{-1}, with a mercury column height of 25 cm, controlled drop time of 1 s and a pulse amplitude of 100 mV (DPP) employing a reference S.C.E. and a Pt auxiliary electrode. The D.M.E. parameters at $h = 25$ cm, were $m = 1{\cdot}50$ mg s^{-1} and $t = 7{\cdot}03$ s in 0·1 mol l^{-1} KCl. The S.M.D.E./H.M.D.E. (Laboratorní přístroje, Prague) was used as a static or a hanging drop electrode. The electrode capillary was 0·128 mm in diameter and the valve was opened for 160 ms to obtain maximum drop size. The supporting electrolyte background was subtracted using a RPT 1 Recording Polarographic Terminal (Laboratorní přístroje, Prague). Oxygen was removed from the solutions by 10-min passage of nitrogen. Nitrogen was purified by passing through an alkaline solution of sodium anthraquinone-2-sulfonate and solutions of chromium(II) ions in diluted hydrochloric acid in contact with zinc amalgam. A gas scrubber with a supporting electrolyte containing an appropriate amount of methanol was inserted between the purification line and the polarographic vessel. The pH was measured with a PHM 62 pH-meter (Radiometer, Copenhagen) provided with a combined glass and saturated calomel reference electrode. Spectrophotometric measurements were carried out on a Specord UV VIS (Zeiss, Jena) using 1 and 2 cm quartz cells.

Procedure

All the calibration curves were measured in triplicate and then subjected to linear regression processing. The detection limit was calculated according to Skogerboe and Grant[12] as the $t \cdot S_{y,x}/a$ value, where $S_{y,x}$ is the standard deviation of the experimental points from the calculated calibration straight line, a is the slope of this line, and t is Student coefficient at the 99% confidence level depending on the number of points used for the construction of the calibration plot. For the extraction-polarographic determination, 5 ml of N,N-dimethyl-4-aminoazobenzene in 0·4 mol l^{-1} hydrochloric acid (50, 5 or 0·5 μmol l^{-1}) was neutralized to pH 7 in a separatory funnel with 2 mol l^{-1} sodium hydroxide. The neutralization was controlled with a universal indicator paper. The solution was then extracted three times with 5 ml of chloroform and the collected extracts were evaporated to dryness at a rotation vacuum dryer. The solid was dissolved in 5·00 ml of the supporting electrolyte containing 50 vol.% Britton–Robinson buffer and 50 vol.% methanol (pH 9·3) and the content of N,N-dimethyl-4-aminoazobenzene was determined by DPP on D.M.E. In the direct polarographic determination of this compound in blood plasma, 1 ml of blood plasma containing a known amount of the substance (added by a microfeeder as a 10 to 0·1 mmol l^{-1} solution) was mixed with 4 ml of the Britton–Robinson buffer (pH 9) and with 5 ml of methanol and was measured by DPP on S.M.D.E. In construction of the linear calibration dependence, methanol containing the required amount of the substance was used.

RESULTS AND DISCUSSION

The stability of the stock solution in methanol was determined spectrophotometrically (1 mol l^{-1}) and by DPP (0·1 and 0·01 mmol l^{-1}). The measurements indicated that the 10^{-3} solutions must be prepared once in 20 days whereas the more diluted solutions should be prepared daily and kept in the darkness. The DPP study of the stability of N,N-dimethyl-4-aminoazobenzene in the studied medium (Table I) revealed that the dilute solutions should be measured at a constant time, immediately after preparation. These conditions should be maintained in measurements of samples used for construction of the calibration plot.

TAST Polarography

With increasing pH, the $E_{1/2}$ values were shifted towards negative potentials. This can be explained by prior protonation of the azo group causing lowering of the electron density in the region of the double bond between the nitrogen atoms. This process facilitates the electron transition from the electrode to the studied molecule. The $\Delta E_{1/2}/\Delta$ pH slope, calculated by the linear regression method, was -85 and -45 mV in the pH range 2–9 and 9–12, respectively. It follows from the slopes of the logarithmic analysis that the process is most reversible in the pH range, 8–11.

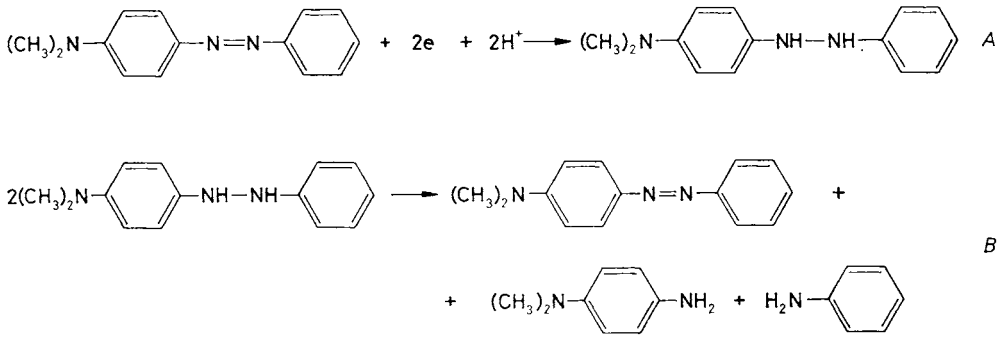
The observed pH dependence of the limiting current indicates that, in an acid medium, transfer of 4 electrons occurs, whereas in alkaline medium only 2 electrons are exchanged. A similar phenomenon was observed in a study of the effect of strong electron-donor substituents (for example OH, NH_2, or $(CH_3)_2N$) on the polarographic reduction of azobenzene derivatives[13,14]. Constant current coulometry[15] demonstrated transfer of 4 electrons for N,N-dimethyl-4-aminoazobenzene over the

TABLE I

Stability of N,N-dimethyl-4-aminoazobenzene in a methanol – Britton–Robinson buffer (1 : 1) mixture. The numerical values give the relative value of the concentration in % compared to the concentration of the freshly prepared solutions

c, mol l^{-1}	pH	t, min				
		15	30	45	60	90
$5 . 10^{-4}$	4·06	100	100	100	100	99
$5 . 10^{-4}$	10·37	100	100	100	100	99
$5 . 10^{-5}$	4·06	100	97	96	95	92
$5 . 10^{-5}$	10·37	99	97	96	95	92
$5 . 10^{-6}$	4·06	98	96	94	92	90
$5 . 10^{-6}$	10·37	99	97	95	93	90

whole pH range. Both these facts can be explained in terms of irreversible disproportionation of a hydrazo compound formed in the first step of the electrochemical reduction (see Eqs (*A*) and (*B*)).



If the disproportionation occurs during the drop lifetime, then the regenerated azo compound can undergo further reduction leading to transfer of 4 electrons. The drop in the limiting current value to about one half, occurring in an alkaline medium, can be explained by a lower rate of disproportionation. At sufficiently high pH, the half time of this reaction is probably one order of magnitude larger than the drop time but is still shorter than the duration of the coulometric measurement which could explain the difference in the number of transferred electrons found by polarography and coulometry. The results of cyclic voltammetry confirmed the irreversibility of the studied process[16]. Higher values of the slopes of the logarithmic analysis than those corresponding to the number of transferred electrons can be explained by a relatively high methanol content. This is consistent with the previously described increase in the irreversibility of the azo group reduction with increasing content of this solvent[17].

The polarographic wave of N,N-dimethyl-4-aminoazobenzene exhibited a maximum, especially in acid medium, that could be suppressed by adding 0·2 ml of a 0·5% solution of gelatine to 10 ml of the analyzed solution. The observed shift of $E_{1/2}$ towards more positive potential values caused by increasing concentration of gelatine may be due to catalysis of the azo group protonation by gelatine that facilitates the reduction[6].

The limiting current was diffusion-controlled when m was $1-5$ mg s^{-1}, t in the range $1-4$ s and pH 4·06 or 9·32.

In TAST polarography at a D.M.E., the dependence of the limiting current at pH 4·06 and 9·32 was linear in the range $5 \cdot 10^{-4} - 10^{-6}$ mol l^{-1}. The determination at pH 4·06 was more sensitive. In the range $(1-10) \cdot 10^{-6}$ mol l^{-1} the slope of the calibration straight line was 6·28 mA mol^{-1} l, its correlation coefficient, 0·9983, and the detection limit, $1 \cdot 10^{-6}$ mol l^{-1}. A further increase in the sensitivity can be

attained by employing TAST polarography at a S.M.D.E., where a concentration range of $10^{-6}-10^{-7}$ mol l^{-1} can be used at both pH values. At pH 4·06 the slope of the calibration straight line was 8·78 mA mol^{-1} l, its correlation coefficient, 0·9990, and the detection limit, $5 . 10^{-8}$ mol l^{-1}. A dependence of $E_{1/2}$ on the depolarizer concentration, most evident at higher concentrations, was also observed, possibly because of adsorption of the depolarizer at the D.M.E. surface[18].

AC Polarography

The AC polarogram of N,N-dimethyl-4-aminoazobenzene (Fig. 1) exhibits a current decrease prior to the peak to a value below that for the supporting electrolyte, indicating the adsorption of the starting compound at the D.M.E.[19]. Because of the irreversibility of the studied system, AC polarography is insufficiently sensitive and its analytical use is limited to the range $(1-5) . 10^{-4}$ mol l^{-1} even for 2nd harmonic current measurements.

Differential Pulse Polarography at a Dropping Mercury Electrode

The dependence of the peak position (E_p) and height (I_p) retained the same character as for TAST polarography. The $\Delta E_p/\Delta$pH slope calculated by the linear regression method was -86 and -60 mV at pH $2-7$ and pH $7-12$, respectively. A decrease in I_p at higher pH values is connected with the decrease in both the number of transferred electrons and the system reversibility (apparent from the slope of the logarithmic analysis of the TAST polarograms).

The observed changes in the peak shape and height with decreasing content of methanol (Fig. 2) may result from the limited solubility of the studied compound in aqueous solutions, its increased adsorption at the D.M.E. and the effect of this solvent on the charge transfer rate. The best developed waves and peaks were obtained with 50 vol.% methanol which was therefore used for all dependences investigated.

In accordance with theory, the peak height and width was found to increase with increasing pulse amplitude and the E_p value became more positive. The peak height was directly proportional to the reservoir height at constant drop time and to the electronically controlled drop time at constant reservoir height.

The dependence of the peak height on the concentration, measured in Britton–Robinson buffer and methanol (1 : 1) at pH 4·06 and pH 9·32 was linear in the $5 . 10^{-4} - 10^{-6}$ mol l^{-1} range. The slope of the calibration plot was 17·5 mA mol^{-1} l at pH 4·06 and 10·1 mA mol^{-1} l at pH 9·32. The correlation coefficient was approximately 0·999. For the region of $(1-10) . 10^{-7}$ mol l^{-1}, 100-fold diluted Britton–Robinson buffer yields a smoother curve for the supporting electrolyte due to lower concentrations of trace impurities in the chemicals used in the buffer. Under these conditions, at pH 4·60 the slope of the calibration plot was 26·8 mA mol^{-1} l, correlation

coefficient 0·9992 and detection limit $1 . 10^{-7}$ mol l^{-1}. A further increase in the sensitivity can be attained in a dilute ammoniacal buffer–methanol (1 : 1) medium at pH 9·72 as the reversibility of the azo group reduction increases in the presence of ammonium salts[4,6]. The calibration plot was then linear from 10^{-6} to 10^{-8} mol l^{-1} with a slope of 31·7 mA mol^{-1} l, correlation coefficient, 0·9958, and detection limit, $1 . 10^{-8}$ mol l^{-1}.

Fast Scan Differential Pulse Voltammetry at a Hanging Mercury Drop Electrode

In agreement with the theory, the peak height increased with the pulse amplitude and the drop size. The measurements were therefore carried out using a pulse amplitude of 100 mA at the largest possible drop size obtained by opening the valve for 160 ms. Optimum results were obtained in a mixture of dilute ammoniacal buffer and methanol (1 : 1), pH 9·72, and polarization rate of 20 mV s^{-1}. Under these conditions, the dependence of the peak height on the concentration was linear in the $10^{-6} - 10^{-8}$ mol l^{-1} range, with a slope of 18·3 mA mol^{-1} l and correlation coefficient of 0·9986. The detection limit, $5 . 10^{-9}$ mol l^{-1}, can be further lowered to $3 . 10^{-9}$ mol l^{-1} using a RPT 1 Recording Polarographic Terminal to subtract the supporting electrolyte curve.

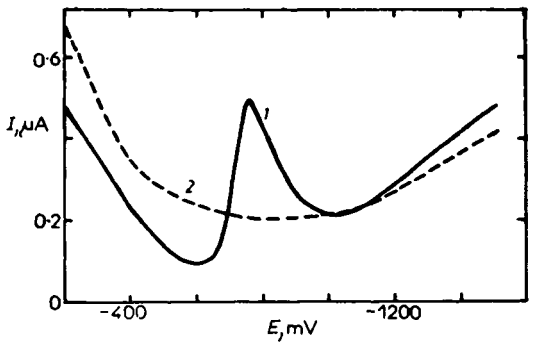


FIG. 1

AC polarogram of $5 . 10^{-4}$ mol l^{-1} N,N-dimethyl-4-aminoazobenzene *1* in methanol–Britton–Robinson buffer (1 : 1) at pH 8·69 and of the supporting electrolyte 2

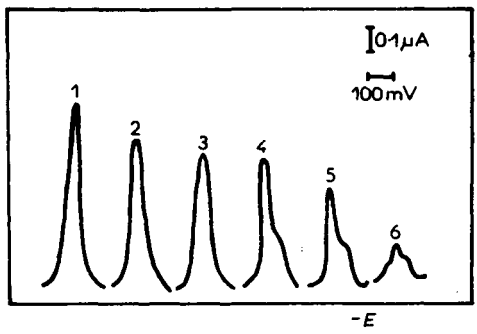


FIG. 2

Effect of methanol on the differential pulse polarogram of $1 . 10^{-5}$ mol l^{-1} N,N-dimethyl-4-aminoazobenzene at a D.M.E. in Britton–Robinson buffer at pH 11·0. The methanol content vol.%: *1* 50; *2* 40; *3* 30; *4* 20; *5* 10; *6* 0·5. Starting potential −100 mV

A sensitivity increase can be based on the fact that the peak height increases with time elapsed between the drop formation and the recording of the curve. (The peak height doubled during 60 s and then remained almost constant. The growth was practically independent of pH and the accumulation potential.) This can be explained by adsorption accumulation of the studied substance at the surface of the drop[10]. For an accumulation time of 60 s at concentration of $10^{-7}-10^{-8}$ mol l^{-1}, the slope of the calibration plot was 32·9 mA mol^{-1} l, correlation coefficient, 0·9989, and detection limit, $2 . 10^{-8}$ mol l^{-1}. The use of accumulation to increase the sensitivity is useful only at concentrations below 10^{-6} mol l^{-1}. At higher concentrations, deviations from linearity occur, probably due to gradual saturation of the electrode surface with the adsorbed depolarizer.

Voltammetry with a Linearly Sweeping Potential at a Hanging Mercury Drop Electrode

The dependence of the peak height on the time elapsed between the drop formation and recording of the curve was similar to that observed for FSDPV. Again the peak height growth almost stopped after 60 s and was independent of the pH and the accumulation potential. In 100-fold diluted ammoniacal buffer–methanol (1 : 1), at pH 9·72 and a polarization rate of 20 mV s^{-1}, at concentration of 10^{-7} to 10^{-8} mol l^{-1}, the slope of the calibration plot was 35·1 mA mol^{-1} l, correlation coefficient, 0·9982, and detection limit, $3 . 10^{-9}$ mol l^{-1}. A disadvantage of this method is a strong dependence of the peak height on the presence of surface-active substances or other compounds that could affect the electrode reaction rate, so the calibration curve method should be employed. If very high sensitivity is not required, the D.M.E or S.M.D.E. is more suitable for the analysis of more complex samples as periodic renewal of the electrode surface minimizes the danger of its passivation.

Extraction-Polarographic Determination

The extraction of organic compounds from aqueous solutions with a suitable organic solvent represents a simple method of separation or preconcentration. The efficiency of the extraction of 10^{-5} mol l^{-1} solutions of N,N-dimethyl-4-aminoazobenzene with chloroform (see Experimental) was spectrophotometrically studied *via* measurement of the absorbance of the chloroform solutions at 415 nm. The calculated degree of a single extraction was 90%. The accuracy and reproducibility of the extraction-polarographic determination, carried out according to the procedure in the experimental part, is illustrated by the following results (average of 5 measurements):

given, μg	56·3	5·63	0·56
found, μg	55·0	5·43	0·56
standard deviation, μg	0·2	0·10	0·03

Direct Determination of N,N-*dimethyl*-4-*aminoazobenzene in Blood Plasma*

Based on preliminary experiments[16], the procedure given in the experimental part was chosen as optimal. Although the polarographied solution was not completely clear and homogenous the concentration dependences were linear in the range of $2 . 10^{-7} - 10^{-4}$ mol l^{-1}. The detection limit was $2 . 10^{-7}$ and $2 . 10^{-6}$ mol l^{-1} in the polarographied solution and the analyzed blood plasma, respectively. The peak height for DPP at a S.M.D.E. in the concentration range of $(2-10) . 10^{-7}$ mol l^{-1} is given by the relationship I_p (nA) = 10·1 . c (μmol l^{-1}) − 1·4 with the correlation coefficient, 0·9974. As the calibration straight line does not pass through the origin, the calibration curve method or method of 2 standard additions should be used. The relative standard deviation of 7 determinations of a $5 . 10^{-7}$ mol l^{-1} solution was 5·2%.

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