POLAROGRAPHIC DETERMINATION OF AZOBENZENE*

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Conditions were found for the determination of azobenzene by means of DC, AC, TAST, DP, and FSDP polarography and linear sweep voltammetry on a hanging mercury drop electrode in the medium of aqueous methanol, which ensures a sufficient solubility of azobenzene. In the latter two methods, the detection limit was around 10^{-8} mol/l; a still lower value could be attained by preliminary accumulation of azobenzene, *i.e.* adsorption on the electrode surface.

Recently, an increased attention has been paid to the polarographic behaviour of azobenzene derivatives[1-4], which is partly due to the carcinogenic properties of many compounds of this type[5,6]. The aim to determine the lowest possible concentrations of azo compounds in biological material led to the use of modern polarographic techniques, especially differential pulse polarography[7-12] (DPP). The polarographic behaviour of azobenzene, especially its reduction mechanism, was studied in detail[4], however the question about its determination in trace amounts by modern polarographic methods remains open. Therefore, we employed TAST polarography, AC polarography, DPP, and fast scan DPP (FSDPP) (ref.[13]) using either the classical dropping mercury electrode or the so-called static mercury drop electrode (SMDE), by means of which the sensitivity is considerably increased, especially in TAST polarography[14]. Further we used cyclic voltammetry, voltammetry with linearly increasing voltage on a hanging mercury drop electrode (HMDE), and preliminary accumulation of azobenzene on the HMDE by adsorption, which was successfully used in the determination of some organic compounds[15].

EXPERIMENTAL

Reagents

A stock solution of azobenzene in methanol (0·001 mol/l) was prepared by dissolving 0·1822 g of the substance (Soyuzkhimexport, Moscow) in 1 l of the solvent: solutions of lower concentration were prepared by dilution with methanol. Ammoniacal and Britton–Robinson buffers were prepared as usual[16] and measured with a digital pH meter. All chemicals were of reagent grade. The solvent was distilled twice prior to use, water was redistilled twice from a quartz apparatus.

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Apparatus

AC polarograms were recorded on an OH 105 type polarograph (Radelkis, Budapest) with a three-electrode system (dropping mercury and saturated calomel electrodes, Pt auxiliary electrode) with the drop time maintained electronically at 1 s and height of mercury column 49 cm. The polarization rate was 400 mV/min and amplitude 10 mV.

TAST polarography, DPP, FSDPP, linear sweep voltammetry, and cyclic voltamperometry were carried out on a PA 3 apparatus with an XY-4105 recorder (Laboratorní přístroje, Prague) and a three-electrode system. Unless stated otherwise, the polarization rate was 2 mV/s, drop time as above, the pulse amplitude in DPP was −100 mV, and a saturated calomel reference and Pt auxiliary electrodes were used. The dropping mercury electrode had a drop time 7·03 s at 0 V in 0·1M-KCl, rate of flow of mercury 1·50 mg/s and height of mercury level 25 cm, which was kept constant unless indicated otherwise. The static or hanging mercury drop electrode was product of Laboratorní přístroje, Prague; the capillary diameter was 0·138 mm, and the largest drop size was used (valve opened 160 ms). The residual current of the base electrolyte was subtracted with Recording Polarographic Terminal RPT 1 (Laboratorní přístroje, Prague). Solutions in the polarographic cell were deaerated by bubbling nitrogen for 10 min, which was purified by bubbling through an alkaline solution of sodium antraquinone-2-sulphonate and a solution of Cr(II) ions acidified with HCl (both in contact with Zn amalgam). Before entering the polarographic cell, the nitrogen passed through a wash bottle filled with the same base solution as in the cell. The solution pH was measured with a PHM 62 type apparatus (Radiometer, Copenhagen) using a combined glass and saturated calomel electrode. A specord UV VIS apparatus, (Zeiss, Jena) and quartz cuvettes of thickness 1 or 2 cm were employed in spectrophotometric measurements.

Method

A 10 ml calibrated flask was filled with 5·00 ml of a buffer solution, a required quantity of the depolarizer and possibly 0·5% gelatin solution, and made up to the mark with methanol. The polarographic curve was recorded 12 min after preparation of the solution. Calibration curves were recorded three times and evaluated by the linear regression method. The detection limit was calculated according to Skogerboe and Grant[17] as described previously[18].

RESULTS AND DISCUSSION

The stability of the stock solution of azobenzene in methanol was checked spectrophotometrically and the more dilute solutions by DPP. The results (Table I) show that the stock solution must be freshly prepared every 10 days and the more dilute ones daily; storage under exclusion of light is preferable. The slow decomposition of azobenzene in the polarographed solution followed by DPP (Table II) shows that the polarographic curves must be recorded soon after preparation of the solution, possibly after a constant time from the preparation has elapsed.

TAST *Polarography on Dropping Mercury Electrode*

The dependences of $E_{1/2}$, I_{lim}, and the slopes of "logarithmic analysis" on pH are given in Table III. The shift of $E_{1/2}$ with increasing pH to negative values can be attributed to a preceding protonation of the azo group resulting in a decrease of the

electron density at the double bond between the N atoms, which in turn results in easier electron acceptance. The slope $\Delta E_{1/2}/\Delta pH$ calculated by linear regression is -77 mV at pH 2–9 and -31 mV at pH 9–12. In agreement with d.c. polarography[1], the slope of the logarithmic analysis increases with pH, the process becoming less reversible. The ratio of the cathodic to the anodic peak height in cyclic voltammetry, their potential difference, and their dependence on the scan rate suggest that the studied process is not quite reversible. The limiting current is practically independent of pH in accord with the fact[19–21] that azobenzene is reduced by the acceptance of two electrons in the whole pH range.

The polarographic wave of azobenzene is, especially in weakly alkaline medium, accompanied by a maximum which can be suppressed by the addition of 0·2 ml of 0·5% gelatin solution. Gelatin causes a shift of the $E_{1/2}$ value to positive poten-

TABLE I

Stability of azobenzene solutions (concentrations in per cent of the initial values)

t, days	%		
	10^{-3} mol l^{-1}	10^{-4} mol l^{-1}	10^{-5} mol l^{-1}
0·5	100·0	99·7	98·3
1	100·0	99·9	97·1
2	100·0	92·1	83·4
5	99·9	85·2	71·3
10	99·8	74·8	62·5
20	99·1	65·4	50·3

TABLE II

Stability of azobenzene in methanol–Britton–Robinson buffer solutions (1 : 1) (compare Table I)

t, min	%					
	$5 \cdot 10^{-4}$ mol l^{-1}		$5 \cdot 10^{-5}$ mol l^{-1}		$5 \cdot 10^{-6}$ mol l^{-1}	
	pH 4·06	pH 10·37	pH 4·06	pH 10·37	pH 4·06	pH 10·37
0	100	100	100	100	100	100
15	100	100	100	99	99	99
30	99	100	98	98	98	99
45	99	100	94	94	96	99
60	98	100	89	89	94	98
90	97	99	76	84	89	96

tials, which can be attributed to catalysis of protonation of azobenzene facilitating its reduction[4].

It was found[22] that the limiting current increases with the height of the mercury level and drop time according to $I_{lim} = km^{2/3} t^{1/6}$, where k is a constant equal to $(0{\cdot}32 \pm 0{\cdot}02)\,\mathrm{nA\,g^{-2/3}\,s^{1/2}}$ for $m = 1-5$ mg/s and $t = 1-4$ s, evidence for diffusion character of the limiting current.

Parameters of the function $I_{lim} = ac + b$ are given in Table IV and the calculated detection limit is given in Table V. The half-wave potential depends somewhat on the depolarizer concentration c, especially above 10^{-4} mol/l. The following values, *e.g.*, were found in the medium of methanol and Britton–Robinson buffer (1 : 1) of pH 4·06

c, mol/l	$5 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-5}$
$E_{1/2}$, mV	−270	−230	−220

This effect may be due to adsorption of the depolarizer on the dropping mercury electrode[23].

TAST *Polarography Using* SMDE

The parameters of the dependence $I = ac + b$ are given in Table IV. The detection limit is much lower than in TAST polarography with a dropping mercury electrode (Table V).

TABLE III

Influence of pH on TAST and DP polarograms of azobenzene (10^{-4} mol/l) in methanol – Britton–Robinson buffer (1 : 1) solutions

pH	$E_{1/2}$ mV	I_{lim} μA	log-slope mV	E_p mV	I_p μA
2·98	−134	0·34	38	−85	0·84
4·06	−233	0·35	44	−175	0·79
5·02	−296	0·36	44	−225	0·80
5·93	−367	0·36	41	−325	0·81
7·04	−451	0·33	44	−425	0·64
8·00	−523	0·32	69	−545	0·49
8·69	−601	0·35	70	−625	0·46
9·32	−634	0·32	71	−660	0·49
10·37	−662	0·36	74	−665	0·52
11·25	−694	0·34	72	−670	0·58
12·08	−704	0·36	62	−675	0·66

AC *Polarography Using* DME

The decrease of the current before the peak below the value corresponding to the base electrolyte (Fig. 1) is evidence for the adsorption of the depolarizer on the dropping mercury electrode[24] mentioned above. The parameters of the concentration dependence are given in Table IV and the detection limit in Table V. The decrease of the slope with increasing concentration is in accord with the theory[24].

Differential Pulse Polarography with DME

The dependence of the peak potential E_p and its height I_p on pH is given in Table III; it has obviously the same character as in TAST polarography. The slope $\Delta E_p/\Delta$pH determined by linear regression is -92 mV for pH 2–9 and -7 mV for pH 9–12. The decrease of I_p at higher pH is related to the decrease of reversibility in accord with the increase of the slope of the logarithmic analysis of the TAST polarograms.

In agreement with the theory[13], it was found that the peak height and width increase with the pulse amplitude, while E_p is shifted to more positive potentials[22]. The peak height is proportional to the square root of the height of the mercury head at constant drop time and increases with the drop time at constant height of the mercury level.

The decrease of the peak height with decreasing content of methanol shown in Fig. 2 is probably related to changing solubility and adsorption of azobenzene and changing rate of the charge-transfer reaction. Equal parts (by volume) of methanol and water are recommended for the purpose of analysis.

Parameters of the concentration dependence are given in Table IV and the detection limit in Table V. Experiments at low concentrations led us to the use of dilute

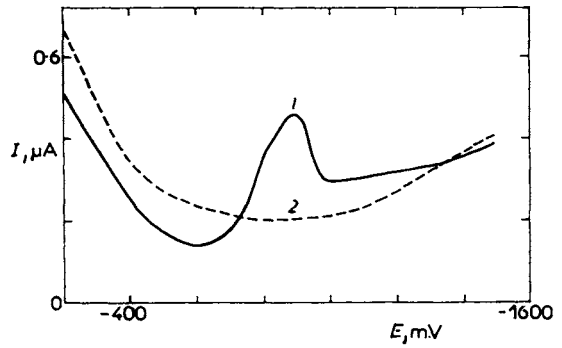

FIG. 1

AC polarogram for $5 \cdot 10^{-4}$ mol/l azobenzene *1* in methanol – Britton–Robinson buffer solution (1 : 1) of pH 10·37 and for the base electrolyte *2*. Current oscillation not shown

TABLE IV

Parameters of the dependence $I = ac + b$ for determination of azobenzene by different methods

Method	c mol l^{-1}	pH	a/Sa[a] mA mol^{-1} l	b/Sb[b] nA	$S_{I,c}$[c] nA	r
TAST/DME	$(1-5) \cdot 10^{-4}$	4·06	3·92/0·11	4/30	60	0·9992
		10·37	3·72/0·08	30/30	50	0·9998
	$(1-10) \cdot 10^{-5}$	4·06	4·15/0·10	3/10	10	0·9991
		10·37	4·12/0·07	−10/10	10	0·9992
	$(1-10) \cdot 10^{-6}$	4·06	3·82/0·16	0/1	2	0·9983
		10·37	3·91/0·20	0/1	1	0·9994
TAST/SMDE	$(1-10) \cdot 10^{-6}$	4·06	5·12/0·08	0·3/0·5	0·7	0·9990
		9·32	4·86/0·07	0·2/0·5	0·6	0·9991
	$(1-10) \cdot 10^{-7}$	4·06	5·08/0·12	0·12/0·08	0·1	0·9978
		9·32	4·70/0·13	0·01/0·09	0·1	0·9969
AC	$(1-5) \cdot 10^{-3}$	10·37	0·15/0·01	170/20	20	0·9985
	$(1-5) \cdot 10^{-4}$	10·37	0·39/0·04	7/14	13	0·9972
DPP/DME	$(1-5) \cdot 10^{-4}$	4·06	8·6/0·1	200/110	20	0·9987
		11·25	6·0/0·1	50/40	50	0·9993
	$(1-10) \cdot 10^{-5}$	4·06	8·9/0·3	29/18	29	0·9994
		11·25	6·0/0·4	27/33	15	0·9993
	$(1-10) \cdot 10^{-6}$	4·06	9·5/0·4	−2/3	5	0·9991
		11·25	7·1/0·2	4/1	2	0·9995
	$(1-10) \cdot 10^{-7}$	4·06	12·6/0·5	−1·8/0·3	0·5	0·9974
		9·72	10·8/0·3	1/0·2	0·3	0·9986
FS DPP/RPT 1	$(1-10) \cdot 10^{-7}$	4·60	12·7/0·3	−0·08/0·2	0·18	0·9992
		9·72	13·8/0·2	−0·04/0·05	0·04	0·9999
	$(1-10) \cdot 10^{-8}$	4·60	12·3/0·5	0·00/0·03	0·03	0·9978
		9·72	16·8/0·6	−0·02/0·04	0·03	0·9983
FS DPP/RPT 1/AK (t_{AK} = 60 s)	$(1-10) \cdot 10^{-7}$	4·60	26·3/0·6	0·8/0·4	0·4	0·9990
		9·72	15·2/1·1	0·9/0·6	0·6	0·9948
	$(1-10) \cdot 10^{-8}$	4·60	28·8/1·1	−0·07/0·07	0·05	0·9980
		9·72	27·9/0·9	−0·11/0·06	0·03	0·9988
LSV/HMDE	$(1-10) \cdot 10^{-7}$	4·60	8·7/0·1	0·1/0·1	0·1	0·9999
		9·72	10·5/0·2	−0·1/0·1	0·1	0·9994
	$(1-10) \cdot 10^{-8}$	4·60	12·4/1·2	−0·02/0·08	0·08	0·9907
		9·72	10·9/0·2	0·02/0·02	0·02	0·9994
LSV/HMDE/AK (t_{AK} = 60 s)	$(1-10) \cdot 10^{-7}$	4·60	11·4/0·6	0·5/0·4	0·3	0·9975
		9·72	15·5/0·6	1·1/0·4	0·4	0·9978
	$(1-10) \cdot 10^{-8}$	4·60	13·6/1·2	0·01/0·01	0·06	0·9930
		9·72	19·2/0·5	0·02/0·03	0·03	0·9991

[a] a/Sa Slope and its standard deviation; [b] b/Sb section and its standard deviation; [c] $S_{I,c}$ standard deviation of experimental points from calculated straight line, r correlation coefficient, t_{AK} time of accumulation.

Britton–Robinson or ammoniacal buffer mixed with methanol (1 : 1) to obtain a less curved line for the base electrolyte. As in TAST polarography, deviations of the calibration curves from linearity and a shift of the E_p value was observed at higher concentration. The slope of the calibration line was appreciably higher when ammoniacal buffer was used. This may be due to a higher reversibility of azobenzene reduction in the presence of ammonium or tetraalkylammonium salts or amines[1,4].

TABLE V
Detection limit for determination of azobenzene

Method	pH	Det. limit (mol/l)	pH	Det. limit (mol/l)
DC	4·06	$3 . 10^{-6}$	10·37	$3 . 10^{-6}$
AC	10·21	$1 . 10^{-4}$		
TAST/DME	4·06	$2 . 10^{-6}$	10·37	$1 . 10^{-6}$
TAST/SME	4·06	$7 . 10^{-8}$	9·32	$8 . 10^{-8}$
DPP/DME	4·60	$12 . 10^{-8}$	9·72	$9 . 10^{-8}$
FS DPP	4·60	$2 . 10^{-8}$	9·72	$2 . 10^{-8}$
FS DPP/RPT	4·60	$8 . 10^{-9}$	9·72	$5 . 10^{-9}$
FS DPP/RPT/AK	4·60	$5 . 10^{-9}$	9·72	$3 . 10^{-9}$
LSV/HMDE	4·60	$2 . 10^{-8}$	9·72	$7 . 10^{-9}$
LSV/HMDE/AK	4·60	$14 . 10^{-9}$	9·72	$5 . 10^{-9}$

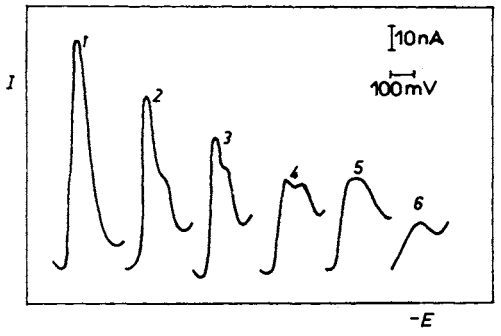

FIG. 2
Influence of methanol on DPP polarograms with dropping mercury electrode for 10^{-5} mol/l azobenzene in methanol – Britton–Robinson buffer solution of pH 4·0. Methanol content in vol.%: *1* 50; *2* 40; *3* 30; *4* 20; *5* 10; *6* 0·5. Starting potential −100 mV

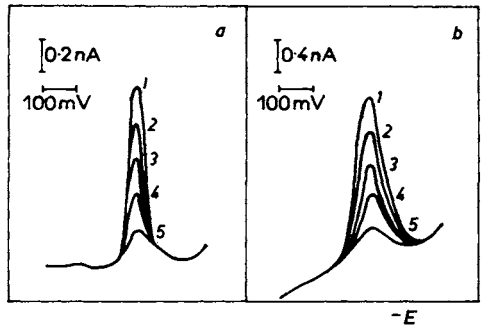

FIG. 3
Linear sweep voltammetry on HMDE for azobenzene *a* without and *b* with accumulation for 60 s. Concentration of azobenzene (μmol/l): *1* 0·1; *2* 0·08; *3* 0·06; *4* 0·04; *5* 0·02. Sweep rate 20 mV/s, base electrolyte dilute ammoniacal buffer (1 : 100) with methanol (1 : 1), pH 9·72. Starting potential −350 mV

Fast Scan Differential Pulse Polarography

The peak current I_p increased with the modulation amplitude and drop size. The results given below were obtained with an amplitude of -100 mV and with the maximum drop size (160 ms opening time of the valve). Furthermore, I_p increased with the time elapsed from the drop formation to the start of the measurement owing to adsorption accumulation of azobenzene on the drop[15]. Based on measurements of the dependence of I_p on the time of accumulation in a quiet solution, the accumulation time 60 s was chosen for analytical purposes. It is apparent from the comparison of the slopes of the calibration lines for FSDPP with or without accumulation (Table IV) that approximately the same increase of sensitivity is obtained at concentrations $10^{-6}-10^{-8}$ mol/l at pH 4·6. At pH 9·7, the sensitivity increased more in the range $10^{-8}-10^{-7}$ than in the range $10^{-7}-10^{-6}$ mol/l. In both cases, the sensitivity increase is independent of the accumulation potential. It can be seen from Table V that the sensitivity can be increased further by using the apparatus RPT 1 for subtracting the residual current of the base electrolyte.

The use of accumulation is purposeful only at the lowest concentrations. Above $6 . 10^{-7}$ mol/l there are deviations from linearity, and the effect of accumulation ceases to be observable around 10^{-5} mol/l. This can be elucidated in terms of the coverage of the electrode surface by the adsorbed depolarizer and equilibrium between its concentration on the surface and in the bulk.

Linear Sweep Voltammetry on HMDE

The method is suitable for the determination of low concentrations of azobenzene; its advantage compared with the pulse methods is that the noise is weaker and the curve of the base electrolyte is less steep. On the other hand, possible passivation by the depolarizer or its reduction products may cause troubles.

A comparison of the slopes of the calibration lines with and without accumulation (Table IV) shows that the increase of sensitivity is higher at pH 9·7 than at pH 4·6, and the increase is more marked in the range $10^{-8}-10^{-7}$ than in the range 10^{-7} to 10^{-6} mol/l. The increase of sensitivity is again independent of the accumulation potential. The voltammograms of azobenzene on HMDE in the range $(2-10) . . 10^{-8}$ mol/l are shown in Fig. 3 for illustration. Of the methods considered, this technique has the lowest detection limit (disregarding the results obtained with the device RPT 1, which is not commonly available).

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